Protein and lipid nutrition of juvenile southern rock lobster, *Jasus edwardsii* (Hutton)

By

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Submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

University of Tasmania

Declaration

This thesis contains no material which has been accepted for a degree of diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of the candidate's knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis

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Abstract

Recent interest in the aquaculture of southern rock lobster *Jasus edwardsii* has developed with increasing demand for lobsters and declining wild fisheries. The potential economic success of lobster aquaculture will depend on the development of efficient formulated feeds specifically designed to meet the nutritional requirements of this species. Experimental lobster feeds are currently based on fish meal and fish oil formulations, and although good survival and growth up to that of lobsters fed fresh blue mussels has been achieved, the potential to increase growth through nutrient level manipulation has not been achieved to date. Investigations of appropriate experimental methods, and subsequent determination of ingredient digestibility, lipid utilisation, histological assessment of energy storage, lipid class and fatty acid composition and energy substrate for metabolism were performed through a series of experiments with juvenile *J. edwardsii*.

Apparent digestibility of protein and carbon from novel dietary ingredients was assessed to screen potential protein sources for dietary inclusion. The apparent digestibility of crude protein in mussel meal (98%), prawn meal (77%), and lupin flour (100%) were higher than the current standard fish meal (63%), and the defatting of fish meal decreased the apparent protein digestibility (53%). Squid (7%) and canola meal (38%) were poorly digested by lobster. The examination of lobster metabolism when fed varying protein levels was measured through oxygen consumption and ammonia-N excretion and their atomic ratio. Lipids were metabolized in feeds containing low protein levels, and carbohydrate and protein were metabolized in high protein feeds, and suggested that carbohydrates were not well utilised.

Isonitrogenous and isolipidic diets were formulated to include a range of potential protein meals and lipid sources, to assess the effect of lipid composition and source on the growth and composition of juvenile lobsters. Fish oil was replaced with vegetable and alternate marine oils in formulated feeds with no significant reduction in weight gain (1.2 - 2.87 g), however lobsters fed the reference feed (fresh blue mussel, *Mytilus edulis*) gained more weight than those fed formulated feeds (5.65 g). Lipid class analysis of the digestive gland lipids indicated high levels of triglycerides (TAG) and diglycerides (DG) in the digestive gland of lobsters fed fresh mussels (FrM), mussel meal (MM) and fish oil with lecithin (FOL). High TAG and DG were associated with digestive gland lipid

storage, and were correlated to high productive protein values ($r=0.755$, $P=0.005$, $n=12$). Lipid histology of the digestive gland confirmed lipid deposition as droplets in R-cells, and the presence of abundant reserve cells in the digestive gland of fast growing lobsters was observed. Dietary phospholipids produced the fastest growth rates in FrM and MM, while dietary triglycerides did not promote growth. Fatty acid composition of lobster digestive gland closely resembled the dietary lipid profile however the predominantly structural lipids in the whole body tissue, more strongly reflected the profiles of control lobsters. The addition of lecithin to fish oil improved the deposition of lipid droplets in the digestive gland.

This study provides greater understanding of the interactions between dietary ingredients and their effects on protein and lipid metabolism, retention and growth and provides valuable information to develop formulated feeds for *J. edwardsii*.

Acknowledgements

I thank my supervisor Prof Chris Carter for his guidance and encouragement throughout this study, and to the all Carter family for their support through the past years. Dr Brad Crear provided facilities, support and research supervision during experiments conducted at the TAFI Marine Research Laboratories. Dr Danielle Johnston introduced me to the marvels of digestive gland histology and her assistance in the histology section of this study is acknowledged. Various research organisations enabled the (as smooth as biologically possible) experimentation and analysis of samples. The School of Aquaculture technical staff, Detlef Planko, Greg Kent and Matt Foale cheerfully assisted with advice on construction and plumbing at the aquatic centre. The aquaculture group at Marine Research Laboratories at Taroona, particularly Drs Arthur Ritar and Stephen Battaglene, provided assistance and facilities for experiments and Alan Beech, Bill Wilkinson, Craig Thomas, Ed Smith and Bob provided access to tools, advice and assistance with all things electrical and aquatic. David Mills, the puerulus collection team and TASSAL provided some animals for experiments. Discussions with Steven Pyecroft at the TAFI Fish Health Laboratories were most helpful regarding lobster histology. The staff at CSIRO Marine Research, particularly Drs Peter Nichols, Graeme Dunstan, Matt Nelson and Matt Bransden provided assistance, facilities and valuable advice with lipid analyses. Drs Peter Nichols, Natalie Moltschaniwskyji and Barbara Nowak generously provided their time and helpful feedback on the final drafts. Carley Bagley, Dr Mark Adams, Robin Katersky, Cynthia Awruch, David Sinn and many others provided assistance throughout this project. Jan, Annabel and Deb were always ready to help, and their assistance on numerous occasions is sincerely appreciated. And most importantly I would like to thank my partner during these PhD years, Justin, for his generous assistance and endless support throughout this project. My family and have always supported my endeavours with enthusiasm and my grandmothers are a source of inspiration and proof that learning never ceases. Without their encouragement, moral and financial assistance throughout my schooling and university studies, this study would not have been possible.

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List of abbreviations

AD_{CP}	apparent digestibility of crude protein
ARA	arachidonic acid
CHO	carbohydrate
CO	canola oil feed
CP	crude protein
DG	diglyceride
DHA	docosahexaenoic acid
EFA	essential fatty acid
EPA	eicosapentaenoic acid
FFA	free fatty acids
FO	fish oil feed
FOL	fish oil and lecithin feed
FrM	fresh mussel (*Mytilus edulis)*
HUFA	highly unsaturated fatty acid
LER	lipid efficiency ratio
LNA	linolenic acid
MM	mussel meal feed
MRL	Marine Research Laboratories, TAFI
MUFA	monounsaturated fatty acid
NH_3-N	ammonia-nitrogen
NSW	New South Wales (Australia)
O:N	ratio of oxygen consumption and ammonia-nitrogen excretion
PC	phosphatidylcholine
PCA	principal component analysis
PER	protein efficiency ratio
PI	phosphatidylinositol
PL	phospholipid
PLV	productive lipid value
PPV	productive protein value
PUFA	polyunsaturated fatty acid
QLD	Queensland (Australia)
SD	standard deviation
SE	standard error
SFA	saturated fatty acid
SQM	squid meal feed
ST	sterol
TAFI	Tasmanian Aquaculture and Fisheries Institute
TAG	triglyceride
TAS	Tasmania (Australia)
TO	tuna oil feed
VIC	Victoria (Australia)
WE	wax ester

Chapter 1

General Introduction

1.1 Aquaculture of Jasus edwardsii

The southern rock lobster, *Jasus edwardsii* (Hutton), is a temperate spiny lobster found in southern Australia and New Zealand. In Australia it is the focus of large commercial fishery worth over A$150 million annually (ABARE, 2003). The development of commercial lobster aquaculture is seen as an avenue to meet the increasing worldwide demand that the declining lobster fisheries cannot supply (Crear and Hart, 2001). Lobster aquaculture is focussed on the palinurid lobsters (spiny lobsters), homarid lobsters (clawed lobsters) and scyllarid lobsters (slipper lobsters) (Phillips et al., 1994; Hooker et al., 1997). Species of decapod crustaceans that are farmed worldwide include freshwater prawns and freshwater crayfish (=crawfish), marine penaeid prawns (=shrimp), crabs and lobsters (Table 1.1). Australian research is focused on three local spiny lobsters; the southern rock lobster, *J. edwardsii,* the western rock lobster, *Panulirus cygnus* and the tropical rock lobster, *P. ornatus.* Semi-intensive spiny lobster aquaculture is established in India, Vietnam, Bangladesh, and the Philippines, and is based on the on-growing of wild caught juveniles in cages and fed on trash-fish feeds. In Australia and New Zealand, it is recognised that future aquaculture will require the hatchery production of puerulus to minimise any potential impact on wild fisheries recruitment and to ensure reliable puerulus supply for industry (Crear and Hart, 2001). While research propagation of *J. edwardsii* has had recent success (A. Ritar, personal communication), short term pilot-farming licences have also been established for the capture of wild puerulus using purpose built collectors (Mills and Crear, 2004). The short-term aim is for puerulus to be transferred to intensive aquaculture facilities. All lobsters in the present study have been captured from the wild in this manner.

Spiny lobsters are characterised by long larval phases which in the wild take up to 2 years (Booth and Kittaka, 1994). This has hampered their hatchery culture to date (Kittaka, 1997). Hatched phyllosoma (larvae) progress through 11 phyllosoma stages and up to 17 instars in open ocean (Ritar et al., 2002), before metamorphosing to the non-feeding nektonic puerulus, which migrates hundreds of kilometres inshore (Lemmens, 1994; Jeffs et al., 2001b). Puerulus settle onto shallow coastal structures (Mills and Crear, 2004) and progress through 3 distinct stages characterised by pigmentation of the carapace and digestive gland and pleopod development (Johnston,

2003) and within 2-4 weeks moult to benthic juveniles and begin feeding (Jeffs et al., 2001a). In Japan and New Zealand lobster aquaculture research has focused on the hatchery propagation of seed stock (Ritar, 2001), husbandry techniques (Crear et al., 2000), disease (Diggles, 1999), environmental requirements (Thomas et al., 2000). The definition of nutritional requirements for *J. edwardsii* and development of effective formulated feeds has been identified as crucial to the success of lobster aquaculture in Australia (Crear et al., 1998; Crear et al., 2000; Smith et al., 2003).

Table 1.1. Decapod crustaceans currently farmed (bold) or candidates for aquaculture world wide. Species of commercial importance in Australia are marked with an asterisk.

Spiny lobster	Crabs
*Jasus edwardsii**	***Scylla serrata****
*Jasus verreauxi**	*Portunus pelagicus**
*Panulirus cygnus**	
Panulirus ornatus*	Penaeid prawns
Jasus lalandii	***Marsupenaeus japonicus****
Panulirus japonicus	***P. monodon****
P. argus	***Fenneropenaeus merguiensis****
P. interruptus	***P. esculentus****
P. guttatus,	***Litopenaeus vannamei***
P. homarus	***P. indicus***
Palinurus elephas	***P. stylirostris***
	P. setiferus
Clawed lobster	***P. semisulcatus***
Homarus americanus	***P. pencillatus***
H. gammarus	***P. chinesis***
Nephrops norvegicus	***P. aztecus***
	P. orientalis
Slipper lobster	***P. schmitti***
Thenus orientalis*	***Metapenaeus macleayi***
*Ibacus peronii**	
	Freshwater crayfish and prawns
	Cherax destructor*
	C. albidus*
	C. quadricarinatus*
	C. tenuimanus*
	Macrobrachium rosenbergii

1.2 Spiny lobster nutrition

Nutrient requirement information for spiny lobsters is very limited and most recommendations are derived from prawn and homarid lobster nutrition. Protein and lipids supply energy, amino acids and fatty acids, and the subsequent requirements reported for nutrients will vary according to the quality of ingredients. This section outlines the current understanding of *J. edwardsii* nutrition with reference to spiny and homarid lobsters, and with recommendations from penaeid crustacean nutrition, where species specific information is not available.

Protein requirements for juvenile *J. edwardsii* have been determined at 29% digestible crude protein (DCP) at 5% lipid, and 31% DCP at 9% lipid (Ward et al., 2003), (equivalent to 35 and 37% respectively, where protein was 83% digestible). Larger *J. edwardsii* grew fastest when fed 36-40% DCP however, the optimum protein level was not clearly defined (Crear et al., unpublished). Protein levels of 49% crude protein (CP) and 6% lipid for *Panulirus cygnus* provided the best growth rates (Glencross et al., 2001), and *P. ornatus* similarly grew faster when provided with high protein feeds (56% DCP, 13% lipid) (Smith et al., 2003). Protein requirements for homarid lobsters vary widely, ranging through 20% (Capuzzo and Lancaster, 1979), 31% (Conklin et al., 1975), 53% (Gallagher et al., 1979) to 60% (Castell and Budson, 1974). The large variation is likely to be due to factors including protein quality, feeding level, dietary energy levels, lobster age, size, stocking, density and temperature. There is no information available on amino acid requirements of *J. edwardsii,* and only two studies in prawns have investigated amino acid supplementation (Chen et al., 1992) and amino acid digestibility of feedstuffs (Akiyama et al., 1989).

The interaction between protein and energy, is suggested to be of more importance than knowing the values of individual nutrient requirements in *J. edwardsii* (Ward et al., 2003), as in other crustaceans (Fox et al., 1994; Cuzon and Guillaume, 1997). The optimal protein/energy ratios for *J. edwardsii* were determined at 29 g DCP.MJ DE^{-1} (Ward et al., 2003), and 28 g $CP.MJ^{-1}$ in *P. cygnus* (Glencross et al., 2001). Similar

ratios were also reported for *P. ornatus* 29.8 g DCP.MJ DE^{-1} (Smith et al., 2003). Carbohydrate has been suggested as an important alternate form of energy for crustaceans (Capuzzo and Lancaster, 1979; Shiau and Peng, 1992), and an investigation into lipid/carbohydrate ratios for *J. edwardsii* suggested a 2:1 ratio (27% carbohydrate and 13.5% lipid) (Johnston et al., 2003).

Lipid requirements in spiny lobsters have not been directly investigated, however good growth rates at relatively high lipid levels have been observed in contrast to penaeid prawns. Fast growth rates have been reported in *J. edwardsii* fed feeds containing 13.5% lipid (Johnston et al., 2003) and Ward et al., (2003) observed higher growth of juvenile *J. edwardsii* at 9% lipid compared to 5%. *P. ornatus* grew fastest with 13% lipid in feeds (Smith et al., 2003), while in contrast *P. cygnus* showed better growth at 6% lipid than 9% lipid (Glencross et al., 2001). Castell (1976) reported high growth rates with 15% cod liver oil in *Homarus americanus* feeds. The successful growth of lobsters on feeds containing high dietary lipids in lobster contrasts with the lower lipid levels (5-8%) recommended for other crustaceans (D'Abramo, 1997). The requirements for fatty acids are detailed in Chapter 6. Briefly, there are three main categories of fatty acids; fatty acids that can be synthesized *de novo* from acetate, odd number straight chain fatty acids (e.g. 19:0, 17:0) and essential fatty acids (EFA's) composed of the linoleic (n-6) and linolenic (n-3) families of polyunsaturated fatty acids (PUFA's). When considering lipid requirements of *J. edwardsii,* it is important to note that marked differences in lipid storage and metabolism occur between different developmental stages. Studies of the condition of wild *J. edwardsii* in New Zealand have shown that the non-feeding puerulus stage derives energy from endogenous phospholipid reserves within haemocoel fat bodies (Nishida et al., 1995), which enable puerulus to maintain clear colouration to avoid predation (Jeffs et al., 2001a). Following settlement, there is a shift to triglyceride storage, and associated development of pigmentation (Jeffs et al., 2001a). Accordingly, care must be taken when comparing the energy utilisation and lipid storage strategies for stages up to puerulus and after settlement as juveniles (Jeffs et al., 2002).

The pigmentation of lobsters fed formulated feeds and mussels, is altered by feeds, and astaxanthin supplementation in feeds for *J. edwardsii* at 115 $mg.kg^{-1}$ was recommended to achieve similar colouration to wild lobsters (Crear et al., 2003).Vitamin and mineral

requirements have not been investigated in *J. edwardsii* and are not well understood in other crustaceans. Requirements for cholesterol, choline and inositol have not been established, but are likely to be essential, and are discussed further in Chapter 6. Formulations for vitamin and mineral premixes follow recommendations from other crustaceans (Conklin, 1997; Davis and Lawrence, 1997).

1.3 Method development: Biological challenges in lobster nutrition studies

To develop formulated feeds for *J. edwardsii,* and to assess their performance, it was necessary to develop feeds that effectively deliver nutrients which are consumed by all lobsters in each treatment. Nutrient delivery is complicated by the biology of lobster, and changes to methods commonly used in finfish nutrition research were required.

Unlike finfish, crustaceans are slow and "messy" feeders. Lobsters locate food by chemosensory detection, and a delay of minutes is normal prior to ingestion. Specialised feeding appendages manipulate and transfer food items to the mandibles, where feed is selectively consumed, fragmented if items are too large to consume, or rejected (Barker and Gibson, 1977). It is during the detection and handling of the feed prior to consumption, where the majority of nutrient leaching occurs. Therefore in nutrition experiments which require accurate measurement of ingested nutrients, it is important to design water stable pellets of the optimal shape for rapid ingestion (Sheppard et al., 2002). Effective binding has been achieved using sodium alginate binders and by including attractants in feeds to aid rapid location and consumption, nutrient leaching from pellets can be minimised (Cuzon et al., 1994). Another source of nutrient loss prior to consumption may result due to the rejection of unsuitably sized ingredient particles (Barker and Gibson, 1977). As feed is ingested it passes to the cardiac stomach where mechanical mastication and primary digestion occurs (Barker and Gibson, 1977; Ceccaldi, 1997). Indigestible or large particles are screened by the filter press and diverted via the ventral groove to the hindgut for faecal expulsion (Gibson, 1983). Therefore in the preparation of lobsters feeds, screening ingredients to a standard particle size prior to pelleting reduces the selective rejection of particles according to size (Cuzon et al., 1994).

Feed intake in spiny lobster in experimental culture units is affected by dominance hierarchies, however, there have been no reports of detrimental effects on growth in communal tanks as opposed to isolated holding (Thomas et al., 2002). Lobsters grow well in communal tanks, and wild juvenile and subadult *J. edwardsii* are found in high densities inside ledges and caves (Edmunds, 1995). To avoid agonistic interactions affecting feed intake in the present study, where possible, feed was distributed evenly around the culture tank. While influenced by agonistic interactions, feed intake of individual lobsters varied independent of hierarchical rank (Thomas et al., 2002), and periods of high feed intake may be followed by several days of low intake (Zoutendyk, 1988; Thomas et al., 2002). Feeding activity peaks at dusk and is high during the first few hours of darkness, with continual feeding during the night (Ward et al., 2003). Lobsters will feed during the day but at a lower rate than during the night (personal observation). Feeding is also dependent on the stage of the moult cycle, where feeding ceases in the days preceding and following ecdysis (Zoutendyk, 1988).

Ecdysis is a physiologically complex and metabolically demanding process resulting in increases in exoskeletal size. Significant changes in lipid composition, water content, digestive gland structure and feeding behaviour are associated with ecdysis. The stages within the moult cycle have been described in several species (Turnbull, 1989; Musgrove, 2000). When comparing chemical composition and digestive gland histology, it is important to select lobsters in the intermoult period (C), where lobsters are actively feeding with tissue growth and nutrient storage occurring. As the premoult stages (D^0-D^4) progress the new exoskeleton is developed and there is an associated mobilisation of energy and nutrients from the digestive gland preceding ecdysis. During the short post-moult period (A) following ecdysis, lobsters do not feed and are soft shelled and vulnerable to predation. The exoskeleton hardens and mineralises through stage B, and lobsters are non-feeding. During this stage energy reserves are at their most depleted prior to the resumption of feeding (Anger, 1987). The expansion of tissues to fill the new exoskeleton is enabled by the uptake of moisture. As new tissue is produced, the percentage of water is replaced by lipids (Cockcroft, 1997; Jussila and Mannonen, 1997). Therefore in the present study, chemical composition is expressed as a percentage of wet weight (unless stated otherwise) to avoid the misrepresentation of concentrations

due to fluctuating tissue water content (Shearer, 1994). Supply of nutrients during the period of reserve accumulation (stage C) is essential for successful moulting and growth, and where insufficient reserves are provided; zero or negative growth in severe cases has been observed (Cockcroft, 1997). Identifying feed components that optimise energy deposition was therefore considered important to ensure effective energy storage to increase moult increment and growth rates and is further investigated in the present study.

Ingested food is digested and nutrients allocated to physiological processes, including energy storage and growth (Jobling, 1994). In juvenile lobster, the energy reserves are stored mainly as lipids within the digestive gland (=midgut gland, hepatopancreas). The digestive gland is well recognised as an important indicator of nutritional condition in crustaceans, and marked depletion of lipid levels during nutritional stress or starvation has been observed (Rosemark et al., 1980; Storch, 1984; Vogt et al., 1985; Ward et al., 2003; McLeod et al., 2004). The digestive gland of southern rock lobster is similar to that described in other crustaceans (Nishida et al., 1995), and is a symmetrical bilobed organ extending throughout the majority of the carapace, consisting of a series of blind ending tubules (Icely and Nott, 1992). Each tubule consists of a single epithelial layer of cells surrounding the lumen, and contained within a basal membrane. There are four distinctive cell types, E-cells (embryonic), F-cells (fibrillar), R-cells (resorptive) and B-cells (blister-like) (Johnson, 1926). These cells are associated with the secretion of digestive enzymes (F-cells), intracellular and extracellular digestion (B-cells), the storage of glycogen and lipid (R-cells) and thus play an important role in the mobilisation of nutrients and energy reserves for energetically expensive processes like growth, reproduction and ecdysis. Some evidence for the depletion of reserve cells in lobster connective tissues at moult has been documented in other Crustacea (Johnson, 1926).

Understanding the apparent digestibility of ingredients provides important information for feed formulation. Digestibility trials in crustaceans are infrequently performed as part of growth trials, and although a few directed studies for particular species have been performed, methods used between different laboratories vary greatly (Smith and Tabrett, 2004). Lobsters egest faeces for about 12 h, beginning about 1 h post-feeding (Ward et

al., 2003; Irvin and Tabrett, 2005). The total collection of faeces from juvenile lobsters was necessary due to the small amounts of faeces produced (Ward et al., 2003). Faecal strands in *J. edwardsii* are encased in a periotrophic membrane and remain intact in the iced faecal collectors for extended periods of time. A maximum of 6 h between faecal collections was recommended for prawns (Smith and Tabrett, 2004). Full faecal pellets sink rapidly to the base of the tank, while empty membranes float in the water column. Water flow in collection tanks was reduced and a gentle hydrocone established using small pumps, to encourage the central settlement of faeces in an ice collector.

1.4 Feed development

Feed producers will be faced with many challenges in production of lobster feeds: a low-cost diet with high water stability and high nutrient availability and that produces rapid growth. Growth of lobsters fed formulated feeds in growth trials is generally lower than the growth obtained by lobsters fed fresh mussels. The reason for the suboptimal growth on formulated feeds compared to that on fresh mussels is not understood.

Protein requirements were determined for *J. edwardsii* (Ward et al., 2003) with fish meal as the protein source, however the optimum growth achieved was lower than when lobsters were fed mussels. Fish meal has been suggested as a sub-optimal protein source for *J. edwardsii* (Sheppard, 2001), and improving the quality of protein (amino acid composition) and providing more digestible protein sources may improve growth rates. Apparent digestibility provides important data for formulation, and aids the selection of highly digestible ingredients for lobster feeds. Providing nutrients at the optimal ratios will optimise growth and further nutrient requirement research is needed to determine optimal lipid and carbohydrate levels. Lobsters fed all formulated feeds consistently contain lower lipid levels than those fed fresh mussels and the potential to improve lipid storage through the incorporation of phospholipid sources e.g. soybean lecithin has been suggested in homarid lobsters (D'Abramo et al., 1980; Gerring, 1992). Established links between digestive gland lipid levels and growth (Cockcroft, 1997), suggest that improved energy provision in feeds could improve growth.

The replacement of fish meal and fish oil in future aquafeeds is will be necessary with declining supply and increasing cost of fish products (Bell, 1998) and has been extensively studied in finfish (Carter et al., 2003; Williams et al., 2003; Jobling, 2004; Kaushik et al., 2004). The substitution of fish oils with plant oils produced a change in fatty acid profile in tissues, however, it is not known whether this occurs by "dilution" or "washout" (Jobling, 2003, 2004). Fish meal replacement in crustaceans has received limited attention (Tidwell et al., 1993; Allan and Smith, 1998; Smith, 1998). Feeding fresh fish, squid and beef heart to adult *J.* edwardsii induced changes to the digestive gland fatty acid composition (Smith et al., 2004). The effect of extended periods of feeding with alternate lipid and protein sources on the health, energy storage and growth rates of juvenile lobsters is not understood. It will be important to consider marketability of lobsters produced with non-marine ingredients, particularly adverse effects on tissue taste, texture, colour and omega3-omega6 fatty acid ratio (Nelson et al., In press).

1.5 Aims of this study

This series of experiments focussed on the development of experimental methods in order to perform and address the following experimental aims:

- determine methods to assess and measure apparent digestibility coefficients for protein ingredients

- determine the effects of alternate oil sources on the growth performance and digestive gland histology of juvenile lobsters
    - investigate relationships between lipid class composition of tissues and growth
    - describe changes in the fatty acid composition of lobster with changing dietary lipid sources

- Formulate experimental feeds based on assessment of ingredients to
    - assess the use of a short-term bioenergetic approach to lobster nutrition studies

- assess potential energy substrates at differing protein levels using O:N ratios

1.6 Notes on this study

Each chapter of this thesis has been prepared as a manuscript for future submission to peer-reviewed journals, therefore there may be some repetition in sections, particularly the materials and methods. Chapters 4, 5 and 6 use lobster samples from Chapter 3, therefore common materials and methods for experimental sections, are referred to in sections 3.3.1 to 3.3.3. Specific methods related to Chapters 4, 5 and 6 are described within the relevant chapters.

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Chapter 2

Apparent digestibility of potential ingredients as protein sources in formulated feeds for the southern rock lobster Jasus edwardsii

2.1 Abstract

Recent advances in defining the protein and protein energy requirements of southern rock lobster, *Jasus edwardsii* using fish meal based feeds have provided baseline information to produce formulated feeds for commercial culture of the species. A range of commercially available potential protein sources was selected and apparent digestibility of crude protein (AD_{CP}) tested *in vivo* using a 70:30 reference feed:test ingredient method. Changes in digestibility over time were assessed for three ingredients over three consecutive week-long sampling periods. The consistency of AD_{CP} over time, validated the measurement of 10 test ingredients over a one week-long period. The ingredients were ranked according to their AD_{CP}; with mussel meal, prawn meal and lupin meal significantly more digestible than squid meal, canola meal and pea meal. The current standard protein source, fish meal, fell mid range (62.5%) among the ingredients tested, and it appears substantial improvements to the current protein quality of lobster feeds may be possible through the incorporation of protein sources with higher AD_{CP}.

2.2 Introduction

The proposed aquaculture of the southern rock lobster, *Jasus edwardsii* will require the production of a formulated feed to optimise growth throughout growout. Research into protein requirements is underway, however little is known about the protein digestibility of ingredients used in requirement experiments in *J. edwardsii* (Ward et al., 2003). The maximum growth rates observed feeding experimental feeds or commercial prawn pellets is still below growth rates of lobsters fed fresh mussels (Crear et al., 2002; Ward et al., 2003). All experimental feeds have used fish meal as the main protein source. However, there is no evidence to suggest that fish meal is the best utilized protein in lobsters, and fish meal may be restricting growth rates in *J.* edwardsii. The critical need to address fish meal replacement in future aquaculture feeds supports the investigation of alternate proteins to meet and increase current growth rates of aquaculture feeds.

Producing the most efficiently utilized feed involves selecting highly digestible protein sources. Measuring the capacity of lobster to digest various ingredients allows an intermediate screening of the potential nutrient value of ingredients to be included in formulated feeds. Although true digestibility more comprehensively accounts for the endogenous losses of nutrients from sources like cell sloughing and periotrophic membranes, these nutrient losses are small and apparent digestibility is the most practical measure of digestibility (Jobling, 1983; Sugiura et al., 1998; Shearer, 2000; Smith and Tabrett, 2004).

The apparent digestibility of a feed may be affected by many factors e.g. anti nutritional factors like phytases, processing level and temperature, antagonistic interactions between feed ingredients, amino acid profile and the digestive enzyme complement of the target species available to digest the nutrients. The increasing cost of fish meal and need to replace the use of marine meals in commercial feeds has seen the incorporation of novel protein sources in many marine species (Akiyama et al., 1989; Ahamad Ali, 1992; Fleming et al., 1998; Smith, 1998). Plant meals such as soybean meal, lupin meal, canola meal, wheat gluten and pea meal have been identified as potential protein sources due to their low cost and relatively high protein levels, although the maximum

inclusions levels may be restricted by anti-nutritional factors such as oligosaccharides, high starch levels, high NSP and lectins (Farhangi and Carter, 2001). Alternate marine protein meals from seafood by-products and processing waste such as squid, prawn and mussel meals also offer potential fish meal replacements.

Digestibility of ingredients for southern rock lobster has not been investigated and there are few published digestibility data from other lobster species. This is partly due to the difficulty involved with performing digestibility studies in crustaceans. The slow and messy feeding characteristics of these animals make high water stability of feeds essential to prevent the loss of nutrients prior to feed consumption. Inert markers traditionally used to calculate the nutrient density gradient from feed to faces have been associated with decreased feed intake and partitioning in the digestive tract (Deering et al., 1996). In particular, the most commonly used digestibility marker, chromic oxide, has been shown to decrease feed intake at inclusion levels above 1%, and shows inconsistent movement through the digestive tract (Leavitt, 1985; Deering et al., 1996). Partitioning of chromic oxide has been observed in freshwater crayfishes *Procambarus clarkii*, (Brown et al., 1986), *Cherax tenuimanus, C. destructor* (Jones and De Silva, 1997) and also in clawed lobster *Homarus americanus* (Leavitt, 1985). More recent nutrient digestibility studies with fish and crustaceans are now using yttrium oxide, ytterbium acetate and cholestane as inert markers at much lower inclusion levels, with more sensitive detection methods to avoid these partitioning problems (Smith et al., 1985; Deering et al., 1996; Ishikawa et al., 1996; Carter et al., 2003). This study consisted of two experiments, addressing a series of aims:

Experiment 1

1. Investigated if apparent protein digestibility changed according to the daily time of feeding (am or pm).
2. Investigated if apparent protein digestibility differed with time across three weeks.

Experiment 2

1. Measured the apparent protein digestibility for a range of potential ingredients in southern rock lobster feeds.
2. Compared apparent digestibility measures using yttrium oxide and ytterbium acetate as the inert digestibility marker.

2.3 Materials and Methods

2.3.1 Experimental animals

Juvenile southern rock lobster *J. edwardsii* were obtained from the Marine Research Laboratories (Tasmanian Aquaculture and Fisheries Institute, Hobart) where they had been maintained under ambient conditions (12:12 photoperiod, 18°C) in a flow-through seawater system. They were fed blue mussel (*Mytilus edulis*) 3 days per week and a commercial penaeid prawn feed (Higashimaru number 12) four days per week. Prior to experimentation at the School of Aquaculture, University of Tasmania, they were held under similar conditions, and fed *Penaeus japonicus* growout feed (Higashimaru #12) 7 days per week in a recirculating seawater system with a flow rate of 1.00 ±0.2 $L.min^{-1}$ giving 100% turnover every 1.5 h, temperature 17.6±0.5°C, salinity 35±1‰. Water quality was measured twice per week and parameters remained within recommended ranges for lobsters (Crear and Allen, 2002).

2.3.2 Feed manufacture

Test protein ingredients were selected from commercially available marine meals and plant sources. Fish meal (South American fish meal, Skretting Australia, Tasmania, Australia) was tested, and a defatted fish meal was produced by double hexane:ethanol (2:1) extraction (Ward et al., 2003). Squid meal and prawn meal were donated by Ridley Aquafeeds, QLD. Powdered blue mussel meal was donated by National Institute of Water and Atmospheric Research (M. Bruce, Auckland, New Zealand). Canola meal, soybean meal and pea meal were from Pivot Agrifeeds, Carrick, Tasmania, Australia. Lupin flour (*Lupinus albus*) was donated by M.C. Croker Pty. Ltd, Cootamundra, New South Wales, Australia. All ingredients used in both test and reference feeds, were passed through a 1 mm screen prior to inclusion.

The reference feed used South American fish meal (Skretting Australia, Cambridge, Tasmania, Australia) as the protein source and South American fish oil (Skretting Australia, Cambridge, Tasmania, Australia) as the lipid source (Table 2.1). Wheat gluten and pre-gelatinised maize starch (Sigma Chemicals, Sydney, New South Wales, Australia) were used to balance the protein and energy levels. Sodium alginate (Kelco International, Sydney) and trisodium phosphate (Ajax Chemicals, Sydney, New South Wales, Australia) were mixed with the dry ingredients to avoid premature calcium binding until the final pellet form was achieved.

Dry components of the reference feed (Table 2.1) were mixed and stored at –20°C. The test protein ingredient was added at 30% to 69.9% reference feed with 0.1% yttrium oxide as an inert digestibility marker (Table 2.2), which was mixed in a Hobart mixer. Fish oil was added and when thoroughly mixed, water was added to form a stiff dough. The dough was passed through a 3 mm die, and the dough strands placed into a 10% $CaCl_2$ setting bath for 3 min, after which the strands were dried at 30°C in an oven. When below 10% moisture, strands were transferred to a -20°C freezer until feeding.

2.3.3 Digestibility experiment

The lobsters (61.0 ± 0.9 g) were randomly allocated in groups of three to each of eighteen conical 40-L experimentation containers, and three tanks were assigned each test feed. Lobsters were fed equal rations to satiation twice daily (0900 h and 1600 h) for a one hour feeding period, after which uneaten feed was removed, and tanks flushed clean.

After 1 week of acclimation to the allocated feeds and tanks, lobsters were blotted dry using paper towel, weighed and returned to their assigned tank. Lobsters were allowed to feed over a 1 h feeding period. After feeding, the tanks were flushed of all uneaten feed and faecal collectors fitted to each tank and immersed in an ice slurry. Faecal collection continued until just before the next feed, when collectors were removed. The faeces were gently tipped onto a 150 μm screen and rinsed into a sample container using chilled distilled water to remove salts (Brunson et al., 1997). All faecal samples were frozen immediately. During experiment 1, three sampling periods were tested, each of 5 consecutive day's duration. During each pooling period, the faeces produced following

the evening (pm) and morning (am) feeds were pooled separately. In Experiment 2, both am and pm faeces over each of the 5 days were pooled into one sample, which was freeze dried to constant weight and stored at –20°C for chemical analysis. As feeding was generally immediate and of short duration, protein loss from pellets prior to ingestion was assumed to be minimal. If exuviae were consumed, or cannibalism occurred, collection ceased for 48 h but normal feeding retained to evacuate digestive tract of non-pellet materials.

2.3.4 Chemical analyses

Nitrogen and carbon were determined by elemental analysis (Leco CHNS-932 using cysteine standard, and BCR Haricotts Bean standard, in Sn crucibles). Crude protein was calculated as N X 6.25. Yttrium was measured by digesting feed and faecal samples in 3 ml HNO_3:H_2SO_4 (2:1) at 90°C until the residue was clear. A 1:5 dilution in distilled water was analysed for yttrium by flame atomic absorption spectrometry (Varian SprectrAA 300), using a yttrium lamp (Varian, Australia) and N_2O/C_2H_2 flame.

2.3.5 Calculations

Apparent digestibility (%) of the reference feed was calculated

$$AD\% = 100 - [100(\%I_{feed} / \%I_{faeces}) \times (\%N_{faeces} / \%N_{feed})]$$

(Maynard and Loosli, 1969) where I is the inert marker and N the nutrient.

Apparent digestibility (%) of the test ingredient was calculated according to (Sugiura et al., 1998) based on the 70:30 ratio of reference feed to test ingredient in each test feed

$$AD_{Ing}\% = (Nutr_{TD} \times AD_{TD} - 0.7 \times Nutr_{BD} \times AD_{BD})/(0.3 \times Nutr_{Ing})$$

Where AD_{Ing} = apparent digestibility (or availability) of nutrients in test ingredient; $Nutr_{TD}$ = nutrient concentration in test feed; AD_{TD} = apparent digestibility of test nutrients in the test feed; $Nutr_{BD}$ = nutrient concentration in the basal feed; AD_{BD}

apparent digestibility of the basal feed; $Nutr_{Ing}$ = nutrient concentration in the test ingredient.

2.3.6 Statistical analysis

Differences in apparent digestibility coefficients (mean ± S.E.) between the feeds were tested for using one-way ANOVA unless otherwise stated. Significant differences were identified using Tukey-Kramer HSD. Significance was accepted at $P<0.05$. Statistical analyses were performed using the software package SPSS version 11.0.0.

2.4 Results

2.4.1 Experiment 1

There was no interaction between the daily sampling time (am or pm) and feed fed on the apparent crude protein digestibility of lupin, pea and reference feeds (AD_{CP}) ($F_{2,5}=0.918$, $P=0.408$). Similarly there was no interaction between the 3 sampling weeks and feed fed on the (AD_{CP}) of lupin, pea and reference feed over the ($F_{2,6}=0.997$, $P=0.421$), and overall differences in AD_{CP} between the 3 sampling blocks were not significant ($F_{2,16}=2.609$, $P=0.085$).

The AD_{CP} of individual ingredients over the 3 sampling weeks was not significantly different in the pea meal and lupin feeds (respectively $F_{2,10}=0.63$, $P=0.557$; $F_{2,17}=0.321$, $P=0.73$), however there was a significant decrease in the AD_{CP} of the reference 1 feed from between first two weeks and the third week of sampling ($F_{2,16}=9.91$, $P=0.002$) (Table 2.3.).

2.4.2 Experiment 2

As there were no differences in AD_{CP} between am and pm of the feeds tested in Experiment 1, and no overall difference over multiple weeks, in Experiment 2 the daily faecal collections from am and pm were pooled over one week. There were significantly different AD_{CP} among the different test ingredients ($F_{12,37}=6.026$, $P<0.001$) (Table 2.4.).

Lupin flour, wheat gluten and mussel meal had the highest digestibility of the feeds, and had significantly higher AD_{CP} than the squid meal, canola meal and fish meal. The squid meal had the lowest AD_{CP} of the test ingredients, with a significantly lower AD_{CP} than the reference feeds and prawn meal. The processing level of the test ingredients had a significant effect on the digestibility of that ingredient. Flours and powdered ingredients had significantly higher AD_{CP} than hammer milled grains and coarse meals ($F_{2,37}$=11.968, P=0.001). The AD_{CP} of the whole diet was lower and less variable than the AD_{CP} of each test ingredient (Table 2.5). The AD_{CP} values determined using Yb_2O_3 were higher than those determined using Y_2O_3 for both feed ingredients (t=2.629, df=61, P=0.011) and test feeds (t=4.960, P<0.000, df=64) (Table 2.5). Similarly the

2.5 Discussion

Differences in the AD_{CP} between the species and between different forms of ingredient are prevalent as the AD_{CP} is influenced not only by form of ingredient: processing, pelleting process, particle size, inert markers and binders used (Tacon, 1996), but also the age of test animal, experimental conditions, duration of acclimation period to feed, faecal collection method. The majority of digestibility data for crustaceans have measured the apparent digestibility of a compound feed; often of complex formulation with combinations of available protein sources. When designing novel feeds, assessing the apparent protein digestibility of individual ingredients gives an indication of their potential nutritional value in feeds. However, protein digestibility of individual ingredients for crustaceans has been measured in only a few studies, in freshwater crayfish (Reigh et al., 1990) and prawns (Forster and Gabbott, 1971; Akiyama et al., 1989; Shiau and Peng, 1992). The present study measures individual ingredient digestibility, to allow comparison to select superior ingredients for future formulation.

Due to the lack of comparable studies in spiny lobsters and the inherent differences in apparent digestibility between species and measurement protocols, the relative AD_{CP} of these ingredients have been ranked in order of protein availability and compared with similar studies using individual ingredients where possible, or compound feeds where

the test ingredient was a predominant protein sources in the formulation. As such this discussion recommends ingredients showing potential with reference to observed results from recent literature. Marine meals are the primary protein source in most aquaculture feeds, and also included as attractants and as sources of lipids, minerals and pigments. Of the marine meals tested, mussel meal was the most efficiently digested of the ingredients (97.5%). Direct comparisons for the same prawn meal used in this experiment were not possible, however the AD_{CP} calculated (77%) suggests a high digestibility, although with the high variability (CV=0.42), considerable caution in interpretation is recommended. Crustacean meals are regularly used in feeds as attractants and as protein sources. High feed digestibility values were reported for *Penaeus monodon* fed protein combinations of shrimp and fish wastes (92.0%), scallop and shrimp wastes (92.5%) and commercial fish meal (source not specified) and shrimp meal (92.8%) and a sardine and lobster processing waste (85.4%) (Sudaryono et al., 1996). A crab protein based purified feed of similar composition to the current reference feed (46.8% protein, 15.9% lipid) for juvenile homarid lobsters had AD_{CP} of 92.6% , where crab protein comprised (Bordner et al., 1983).

Fish meal is the main protein source used in aquaculture feeds, and experimental rock lobster feeds to date. There is no data for the ingredient digestibility of fish meal in *J. edwardsii*. The fish meal digestibility measured in this study (62.5%) was slightly lower than AD_{CP} observed in an ingredient digestibility study in *P. monodon* 77.5% (Smith and Tabrett, 2004) and in *P. setiferus* where a feed containing menhaden fish meal had AD_{CP} of 75.9% (Brunson et al., 1997). The fish meal digestibility is lower than previously measured protein digestibility for *J. edwardsii* using a compound fish meal based feed (80.3%) (Ward et al., 2003), which may partly be due to the higher digestibility of other feed ingredients. The hexane:methanol fat extraction of fishmeal slightly reduced the digestibility of the resultant de-fatted fishmeal (53.0%). The apparent digestibility of squid meal was surprisingly low (7.3%), which was confirmed by minimal growth in a subsequent growth trial using the same meal (Chapter 3). Total volatile nitrogen analysis of this meal suggested levels above industry standard present and probable poor batch quality is the likely cause of the low digestibility. Squid meal in other crustacean studies has relative high digestibility.

Plant materials are increasingly being used to replace fish meal in aquaculture feeds as they are readily available, inexpensive and with protein levels suitable for formulating feeds. The pulses, field peas and lupins and oil seeds such as soybean and canola are available in various forms and stages of processing and as protein concentrates and isolates for inclusion in aquaculture feeds. Meals in this study were commercial grains processed to a uniform level, rather than high protein concentrates as used in many studies. Of the plant meals in the present study, the lupin flour had the highest apparent digestibility with a value of 100.1%. This compares well to a study by (Smith, 1998) in *P. monodon* where a dehulled lupin meal protein was 95% digestible.

Cruz-Suarez et al., (2001) reported 89.0% AD_{CP} for a whole raw pea meal in *Litopenaeus stylirostris* and (Bautista-Teruek et al., 2003) similarly reported 84.4% AD_{CP} in *P. monodon*. The high processing levels of the reported meals may have increased the amount of protein digested when compared to the present study, using a twice hammer milled pea meal, which had a protein digestibility of 52.0%. Soybean meal in the present study was also hammer milled twice, and had a digestibility value of 60.5%. Although variable, the AD_{CP} appears far lower than the reported value of 92.0% for *P. monodon* (Smith, 1998). Canola meal was not well digested in rock lobster (38.3%), which contrasts to reported studies in *P. monodon* which had 78.0% AD_{CP} (Smith, 1998). Wheat gluten was also very well digested (90.1%) by rock lobster. *P. monodon* have been reported to digest wheat gluten well also with 102% digestibility (Smith, 1998). High grade wheat flours have been used in feeds for *P. monodon* with protein digestibility of around 80% (Shiau et al., 1991).

The present study aimed to determine the apparent protein digestibility of individual ingredients; however, it is worthy to note that the test ingredient apparent digestibility increased was more variable than the AD_{CP} and AD_{C} of whole feeds, where the replication error between samples was amplified in the test ingredient calculation. The digestibility of the whole feeds are within ranges previously reported for whole feed digestibility where the AD_{CP} for feed containing mussel meal (83-90%) was similar to the 91%AD_{CP} for feeds containing mussel mantle meal in *P. platyceros* (Forster and Gabbott, 1971). A recent study in the spiny lobster *P. ornatus* fed feeds containing krill meal, krill hydrolysate and fish meal measured 91% AD_{CP}, which was higher than whole

feed AD_{CP} in the present study. However, the fish meal based feeds (75 -78% AD_{CP}) were similar to those obtained for white fish meal based feeds for *P. platyceros* (Forster and Gabbott, 1971).

The AD_{CP} determined using Y_2O_3 were significantly higher than those determined using Yb_2O_3, which is in contrast to a previous study in salmonids where there was no difference between AD_{CP} determined using Y_2O_3 or Yb_2O_3 (Austreng et al., 2000). Two studies have higher AD_{CP} using Yb_2O_3 in *P. monodon* than chromium oxide (Deering et al., 1996; Smith, 1998). The levels of ingredient processing especially for plant proteins should also be examined further to assess what level of processing is necessary to enable sufficient digestibility. The filtering and rejection of large particles from ingested homogenates is well documented, and to avoid the selective rejection of feed components ensuring that the particles suit the size of the animal to be fed is essential. Further trials to examine the ingredient particle size effect of digestibility are underway for this species.

From this study, it appears that the marine meals; mussel meal and prawn meal are more digestible than the fish meal currently being used. This suggests there is considerable potential to increase growth and further trials to quantify the nutrient value of these ingredients over long term growth experiments are warranted. Recent growth experiments comparing fresh mussels and mussel meal based feeds have supported superior growth when compared to fish meal based feeds; the standard used for formulated commercial and experimental feeds (Crear et al., 2002; Ward et al., 2003). The potential to increase the growth observed in growth trials to date is suggested. Considerable potential to replace fish meal with plant proteins was evident especially with the lupin, wheat gluten and soybean meals, however these must also tested for longer terms growth and health effects in this species. Further examination of the processing level of these plant ingredients may enhance the digestibility values reported and provide potential partial marine meal replacements in future feeds. Further studies to determine AD_{CP} using a range of inert markers in *J. edwardsii* is warranted. In the present study the current method allows comparison to select potential ingredients from the range of AD_{CP} determined. Determining apparent digestibility values provide a valuable initial step at the start of the ingredient evaluation process, preceding

assessment of the amino acid profiles, presence and effects of antinutritional factors, and the measurement of long term growth rates, health and survival of the lobsters.

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Table 2.1. Ingredient composition of the reference feed $g.kg^{-1}$

Ingredient	Inclusion
Fish meal	639.67
Pre-gelatinised maize starch	143.54
Wheat gluten	120.00
Fish oil	30.00
Manucol	46.77
Trisodiumbisphosphate	20.00
Water	As required*

* water added at about 40% to form a soft dough for extrusion

Table 2.2. Ingredient composition of the digestibility test feeds g.kg^{-1}

Ingredient	FM	DFM	PRM	SQM	MM	CM	SBM	LMA	PEA	WG	REF1	REF2	REF3
Reference feed	698	698	699	699	698	699	699	699	699	698	999	999	998
Yttrium oxide	1	1	1	1	1	1	1	1	1	1	1	1	1
Ytterbium acetate	1	1			1					1			1
Test ingredient													
Fish meal	300												
Defatted fish meal		300											
Prawn meal			300										
Squid meal				300									
Mussel meal					300								
Canola meal						300							
Soy bean meal							300						
Lupin meal								300					
Pea meal									300				
Wheat gluten										300			
Chemical composition g.kg^{-1}													
Crude protein													
Test ingredient	656.3	692.5	318.1	691.3	536.9	393.8	475.0	373.1	223.1	725.6			
Test feed	554.4	565.6	452.5	564.4	519.4	475.6	499.4	469.4	423.8	575.6	510.6	510.6	511.9

Table 2.3. Percentage apparent protein digestibility coefficients (AD_{CP}) for various ingredients fed to juvenile Jasus edwardsii over a three one-week sampling periods (after 1 week of feed acclimation).

Feed	Sampling week	AD_{CP}	S.E.
Lupin meal	1	104.84	6.9
	2	97.79	5.9
	3	97.73	6.3
Pea meal	1	69.01	26.0
	2	54.40	31.0
	3	32.54	8.3
Reference feed	1	96.94 [a]	5.5
	2	80.84 [a]	9.7
	3	54.51 [b]	4.2

Significant differences are indicated by different superscripts

Table 2.4. Percentage apparent protein digestibility (AD_{CP}) of various marine protein and plant protein sources for juvenile southern rock lobster J. edwardsii over a week sampling period.

Feed	AD_{CP}	S.E.
Marine protein sources		
Mussel meal	97.64	0.1
Prawn meal	77.21	19.1
Pivot fish meal	62.52	1.4
Defatted fish meal	53.08	5.7
Squid meal	7.26	2.3
Plant protein sources		
Lupin flour	100.12	6.0
Wheat gluten	90.11	9.7
Soybean meal	60.53	19.0
Pea meal	51.98	8.7
Canola meal	38.34	13.7
Reference 1	77.43	9.02
Reference 2	70.31	4.61
Reference 3	75.00	10.74

Table 2.5. Percentage apparent protein digestibility (AD_{CP}) of test feed (70% reference feed 3 and 30% test ingredient) and test ingredient for juvenile southern rock lobster *J. edwardsii* over a week sampling period

	Test feed (AD_{CP})				Test ingredient (AD_{CP})			
	Yb_2O_3		Y_2O_3		Yb_2O_3		Y_2O_3	
Test ingredient	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Defatted fish meal	74.11	±1.24	74.44	±2.10	74.91	±3.37	59.20	±5.72
Mussel meal	82.89	±1.95	90.18	±0.04	103.47	±6.28	105.54	±0.14
Fish meal	75.23	±1.53	78.22	±0.49	78.12	±4.33	68.99	±1.39
Reference 3	73.64	±6.23	83.28	±3.22	73.64	±20.76	83.28	±10.74
Wheat gluten	86.47	±3.4	90.70	±3.37	107.57	±9.00	102.91	±8.92
Starch	68.87	±1.83	91.92	±0.27	-166.00	±92.06	512.77	±13.66

Chapter 3

Growth and body tissue composition response to different oil sources in rock lobster feeds

3.1 Abstract

Experimental lobster feeds are currently based on fish meal and fish oil formulations, and although good survival and growth up to that of lobsters fed fresh blue mussels has been achieved, varying protein levels in feeds has not increased growth to that of natural food. This experiment assessed the performance of a range of oils and oil containing ingredients (fish oil, fish oil with added lecithin, canola oil, tuna oil, mussel meal, squid meal) in their commercially available form in lobster feeds. Performance was assessed by growth rate, survival, final chemical composition and gross nutrient retention and efficiency. Groups of 15 post-larval lobsters were randomly allocated one of six test feeds in triplicate. Two remaining tanks were fed freshly opened blue mussels. Lobsters were fed daily to excess, and weight measured every three weeks. At the end of the experiment final weights of whole body and digestive gland were measured. There were no significant differences in survival, weight gain or specific growth rate among the lobsters. However, the squid meal fed lobsters had a significantly lower lipid efficiency ratio and lipid productivity value than for all other feeds. Canola oil fed lobsters had low lipid retention within the digestive gland. Mussel meal and tuna oil fed lobsters had the closest digestive gland chemical composition most similar to that observed in the fresh mussel fed animals. There was no significant reduction in growth of lobsters fed alternative lipid sources. The substitution of fish oil and fish meal with alternative ingredients produced a range of growth rates with the lowest growth in lobsters that were fed squid and canola based feeds, and the fastest growth was achieved by including tuna and mussel ingredients into feeds. Further work identifying the mechanisms for the storage and metabolism of different dietary lipids is warranted, as is the examination of different inclusion levels and combinations of new ingredients to improve current lobster feeds.

3.2 Introduction

The high-value southern rock lobster *Jasus edwardsii* (Hutton, 1875) supports economically important fisheries in Australia and New Zealand (Jeffs and Hooker, 2000; Stevens and Sykes, 2000). Expanding consumer markets and fully exploited wild fisheries have motivated recent interest in the aquaculture and enhancement of this species. Aquaculture research to investigate the environmental, health and nutritional requirements of this species is underway (Crear et al., 2000; Ward et al., 2003). Commercial pilot farms based on the growout of wild-caught pueruli are operating in Tasmania, New Zealand, India and Vietnam. In Tasmania, puerulus capture is exchanged for a percentage of adult fisheries quota, and set percentages of juveniles are released into the wild to equate to wild mortality rates, to ensure biological neutrality (FRDC, 2000). Currently these experimental farms rely on sole or supplementary feeding of blue mussels, *Mytilus edulis,* to sustain high growth. Prohibitive mussel prices in Australia exclude the blue mussel as a viable long-term feed, and the development of a sustainable specific formulated feed for rock lobster is seen as a research priority (FRDC, 2000; Jeffs and Hooker, 2000).

Production of aquafeeds relies on the inclusion of marine meal and oils, in particular fish meal and fish oil. Fish oils are produced for the human pharmaceutical industry and as human dietary additives (Nichols et al., 2004). Lower grade fish oils have been traditionally used in aquafeeds, but increasing fish meal and oil prices, and a need to find alternatives to wild fish products has prompted new efforts to replace fish products to maintain the economic sustainability of aquafeeds (Bell, 1998; Carter and Hauler, 2000; del Mar Otero-Villanueva et al., 2004). The replacement of fish oil with alternative marine oils, waste processing products containing oils and the partial substitution of fish oil with plant oil sources, are potential avenues meet lipid requirements of cultured species (Bell, 1998; Bell et al., 2003; Carter et al., 2003). Preliminary data from nutrient requirement studies in cultured rock lobster have shown that fresh mussels support the best growth (Crear et al., 2000; Crear et al., 2002; Glencross et al., 2001; Tsvetnenko et al., 2000; Ward et al., 2003). The lobsters fed formulated fish meal-fish oil based feeds in comparison exhibited both slower growth and poor lipid storage in the digestive

gland, suggesting inadequate lipid provision, possible due to inappropriate lipid sources or inclusion levels (Ward et al., 2003).

Dietary oil provides essential fatty acids which are integral in cell membrane structure and provide components and precursors for the synthesis of hormones that are required for many cellular processes (Sargent, 1976). Lipids provide a large proportion of the energy in the diet required to fuel metabolic processes. The crude lipid requirements of crustacean species are generally lower than marine and freshwater fishes, ranging between 4-13% lipid (D'Abramo, 1989), and in lobster up to 18% lipid has been fed with no adverse effects on survival (Gerring, 1992). However, when reporting crude lipid requirements, it must be recognised that the requirements for the various essential fatty acids may be met through different ingredient lipid profiles, and recommendations remain a guideline for that ingredient. Lipids have been recognised as important energy sources in various life stages of the rock lobster (Jeffs et al., 1999; Jeffs et al., 2001; Jeffs et al., 2002; McLeod et al., 2004), and are preferentially metabolised before carbohydrates and proteins in larval *J. edwardsii* (Jeffs et al., 1999). Starvation experiments have been widely used to determine the relative changes in chemical composition over time (McLeod et al., 2004; Smith et al., 2003); however induced starvation compared to the *ad libitum* provision of feeds with an inadequate nutritional profile present two different nutritional states which are unlikely to generate similar metabolic responses. The chemical composition of any organism is the product of dietary intake and subsequent metabolism (Sargent, 1976). When considering dietary crude lipid requirements; the quality and concentration of the fatty acid profile and the dietary energy content may both influence the level of lipid storage and biosynthesis and growth (D'Abramo, 1997). Understanding the factors driving variation in lipid profiles in order to optimise the provision of lipids at maximum growth rates is of importance to further improve the dietary lipids. The importance of lipid nutrition in larval and juvenile *J. edwardsii* had been recently emphasised (Jeffs et al., 1999) and the association between poor growth and poor lipid retention described in previous studies (Cockcroft, 1997; Ward et al., 2003) warrant the further investigation of lipid utilisation from feeds.

Although the importance of lipid as the primary energy source beyond the larval stages has yet to be established, lobsters exhibiting slow growth in past experiments also exhibited abnormalities with lipid storage, digestive gland lipid content and appearance (Ward et al., 2003). Poor lipid deposition in the digestive gland through the intermoult phase may reduce the potential for growth, and in extreme cases of malnutrition, the ability of the lobster to successfully complete ecdysis (Cockcroft, 1997). Past literature has described restricted lipid transport in lobsters and prawns fed feeds without lecithin (Conklin et al., 1980). Coutteau's (1997) review of lecithin use in crustacean feeds emphasised the benefits of lecithin inclusion on growth and survival as either crude lecithin or a purified phospholipid source. Lecithin has been suggested to improve growth rates and survival at inclusion levels between 0.5-7.5% for various species, however the dietary requirement for *J. edwardsii* has not been established. In the current study, crude soybean lecithin was partially substituted for fish oil to investigate further compositional and growth effects that may benefit rock lobster feed formulations. This study will examine the effect of different oil sources on lobster growth performance and efficiency with the aim to optimise the provision of lipids in lobster feeds.

3.3 Materials and Methods

Rationale for experimental design: nutrient efficiency versus nutrient productivity?
This experiment investigated the utilisation of ingested nutrients for net growth or for catabolism according to changes in the lipid component of each feed. Nutrient efficiency ratios describe the conversion of consumed nutrient into weight gain. Weight gain could occur via the catabolism of ingested lipids and proteins to provide energy, free fatty acids and amino acids for incorporation into new biosynthetic compounds and tissue growth. Alternatively nutrient productivity describes the proportion of ingested nutrient that is retained in the body. Where dietary nutrients ingested are not catabolised for biosynthesis they may be excreted, defecated or stored as longer term fuel depots. The magnitude of catabolism, excretion or storage can be partially explained by the protein and lipid productive value. During catabolism and tissue growth, dietary nutrients are retained as the same nutrient within the body, while changes in proportions of nutrients

may indicate excretion or deposition of nutrients. The difference between these two indices; productive value and growth efficiency ratio leads toward some understanding of the feed potential to provide for nutrient deposition as growth, or the flux between nutrient storage or excretion.

Feeds were formulated to provide a range of lipid containing ingredients (oils and lipid-rich meals) with potential for commercial use in feeds in their available form. Of interest was the growth response and lipid retention within the digestive gland and the relationship to dietary lipid source. Individual data were analysed to allow the investigation of dietary lipid effects on the chemical, lipid class and fatty acid composition and on the digestive gland histology of the cultured lobsters.

3.3.1 Feeds

Feeds were made at the School of Aquaculture, University of Tasmania. Feeds were formulated to be isonitrogenous, isolipidic and isoenergetic, but to contain different lipid sources; 4 purified oil sources and 3 oil-containing marine meals (Table 3.1). The lipid sources were cold pressed canola oil (Golden Fields, NSW), South American fish oil (Skretting Australia, TAS), tuna oil (Hi-DHA®, donated by Clover Corporation, VIC), a combination of soy lecithin (Sigma Chemicals, NSW) and the fish oil (Skretting Australia, TAS). The marine meals: South American fish meal (Skretting Australia, TAS), squid meal (Ridley Aquafeeds, QLD) and powdered blue mussel (National Institute of Water and Atmospheric Research, New Zealand) were incorporated at 450 $g.kg^{-1}$. Pre-gelatinised maize starch (BO11C, Sigma Chemicals, NSW) and 70% vital wheat gluten (Sigma Chemicals, NSW) were used to balance the energy and protein levels of the feeds. A vitamin premix was made from individual vitamins (Sigma Chemicals, NSW) according to D'Abramo (1997), Stay C vitamin C and carophyll pink (Roche Australia, NSW), were included with the antioxidant Banox E (Sigma Chemicals, NSW). The alginate binder Manucol DM (Geraldton Industries, NSW) was combined with trisodiumpyrophosphate (TSPP) (Ajax Chemicals, NSW) to avoid premature calcium binding until the final pellet form was achieved.

The dry materials were thoroughly mixed in a Hobart mixer for 1 h. Fish oil was added and mixed for 30 min. Sufficient distilled water was added to form a firm dough, which

was then passed through a 3 mm die on a Kenwood mincer attachment. Pellet strands were immersed in a 10% $CaCl_2$ bath for 3 mins to set and then dried at 30°C. When below 10% moisture, strands were transferred to a -20°C freezer until feeding. Dried pellets (<10% moisture) were broken into about 1.5 cm lengths and stored at –20°C until use.

3.3.2 Experimental lobsters

Juvenile rock lobster *J. edwardsii* were caught using puerulus collectors on the east coast of Tasmania, (August 2001), and transported to the Marine Research Laboratories (Tasmanian Aquaculture and Fisheries Institute) where they were maintained (ambient photoperiod, 18°C) in three flow-through 400-L seawater tanks. They were fed blue mussels (*Mytilus edulis*) 3 days per week and a commercial penaeid prawn feed (Higashimaru No.12) 4 days per week. The lobster puerulus (non-feeding) were held under these conditions with access to feed until all had developed pigmentation indicating post-larval metamorphosis, and until they accepted pelleted feed. Water quality was measured weekly and managed to control quality to within the recommended ranges for lobsters (35 ppt, pH 8.4, nitrate <10mg.L^{-1}, nitrite <0.1 mg.L^{-1}) (Crear and Allen, 2002). Tanks were maintained at ambient photoperiod of approximately 12L:12D, at 18°C ± 1.5.

3.3.3 Experimental protocol

Fifteen lobsters were placed into each of 20 400-L tanks. Six formulated feeds were each allocated to 3 tanks, and 2 tanks were fed freshly opened mussels. Lobsters were fed to satiation for 7 days of acclimation after which lobsters were blotted dry using paper towel, weighed and returned to their assigned tanks to begin the growth experiment. Lobsters were fed once daily at 1700 h to satiation and uneaten feed removed between 0900 and 1000 h each morning by siphon onto a 150 μm screen. Uneaten feed was stored at –20°C until each three-weekly weight check, and then dried at 50°C for 48 h and weighed. The 2 mussel-fed treatments were fed 5 blue mussel halves each, which were removed at 0900-1000 h. Weight checks of total tank biomass were performed at weeks 3, 7 and 10. Mortalities were removed and the date recorded. Tank floors were siphoned of debris every other day, and tank walls, hides and standpipes cleaned weekly.

At the conclusion of the experiment, feeding ceased 24 h prior to sampling in all tanks. All lobsters were dried and weighed as above, then the five most median sized lobsters in the intermoult stage (Turner, 1989) from each tank were killed in an ice-water slurry, and their digestive glands were immediately removed. Four of the digestive glands were bisected longitudinally, with half was rapidly frozen in liquid nitrogen for chemical and fatty acid analysis and half fixed for histology. Two (half digestive glands from two individual lobsters) were fixed in 2.5% glutaraldehyde overnight and postfixed in osmium tetroxide for lipid histology, and two in Gendre's fluid for carbohydrate histology. The remaining whole digestive gland was rapidly frozen in liquid nitrogen for proximate and fatty acid analysis.

3.3.4 Chemical analyses

Nitrogen was determined by Kjeldahl digestion in H_2SO_4 with Cu/Ni catalyst, and liberated ammonia was measured using the Kjeldahl distillation unit, and titrated against 0.56 M HCl. Crude protein was calculated as nitrogen X 6.25. Energy was measured by complete combustion in a Gallenkamp bomb calorimeter. Where insufficient tissue was available to make a pellet, gross energy was calculated using values protein=35.45 $J.g^{-1}$, carbohydrate=17.95 $J.g^{-1}$, lipid=39.54 $J.g^{-1}$ (Blaxter, 1989). Total lipid was determined gravimetrically using a modified chloroform:methanol extraction (Bligh and Dyer, 1959). Ash was determined after combustion in a muffle furnace for 16 h at 550°C. Carbohydrate was determined by difference of total dry weight from the sum of protein, total lipid and ash (AOAC, 1990).

3.3.5 Calculations

Whole body chemical composition was calculated according to the percentage composition and relative weights of both the digestive gland, and whole body without digestive gland. All chemical composition values of lobster tissues are presented as a percentage of the wet weight of the lobster to avoid compromised data where tissue lipids are replaced with water (Shearer, 1994). Feed composition is expressed on a dry matter basis.

Nutrient efficiency ratio for protein (PER g.g $protein^{-1}$) and lipid (LER g.g $lipid^{-1}$) was calculated as: g weight gain / g nutrient consumed*100. The productive nutrient value

for protein (PPV %) and lipid (PLV %) was calculated as: g nutrient weight gain / g nutrient consumed*100. Specific growth rate (%.d^{-1}) was calculated as: ln(final wt)-ln(initial weight)/days*100.

3.3.6 Statistical analysis

The normality and homogeneity of data were explored by examining residual plots. As required, data were transformed using arcsine transformation for percentage data, and square root or $\log_{10}$ transformation for non-percentage data. Where transformation did not reduce variation or skew, data were left untransformed. One-way analysis of variance was used to determine significance at α=0.05 unless otherwise specified. Tukey's HSD was used to determine significant differences between groups using SPSS software version 11.5. Lobsters in three adjacent tanks fed squid meal (SQM), fish oil and lecithin (FOL) and tuna oil (TO) were affected by a *Vibrio harveyii* infection in the last week of the experiment, which caused the death of smaller lobsters in these tanks. These tanks were omitted from all statistical analyses and subsequent discussion.

3.4 Results

The weight gain of lobsters fed the formulated feeds ranged between 1.23 g and 2.87 g for lobsters with FOL fed lobsters gaining significantly more weight than SQM fed lobsters (F=5.057, df=5, P=0.038). Formulated feeds produced a significantly lower average weight gain (2.32 ±0.2 g) than the fresh mussel (FrM) fed lobsters (5.65±0.1 g) (t=-6.717, df=15, P<0.001) (Table 3.2). The survival of lobsters was not significantly different between the dietary treatments (F=0.819, df=5, P=0.565), the mortality observed was due to cannibalism. Squid meal (SQM) produced a significantly lower mean SGR (0.87±0.3 %.d^{-1}) than all other formulated feeds (1.40±0.07 %.d^{-1}), while among the formulated feeds mussel meal (MM) and the fish oil and lecithin (FOL) produced the fastest SGR. Feed intake between the feeds was not significantly different (F=1.57, df=5, P=0.262). There was no significant difference in DGI between feeds (t=2.076, df=15,2, P=0.056).

3.4.1 Whole body

The whole body crude protein was highest for the feeds FOL and MM at 13.5% crude protein. The TO, CO, FO fed lobsters all contained close to 12% crude protein, similar to the fresh mussel fed and initial lobster composition (12.3% and 12.2%) respectively while SQM had significantly less crude protein in the whole body at 10.1% (Table 3.3) Dry matter levels in the whole body tissues were not significantly different among the formulated feeds ($F=2.850$, $df=5,1$, $P=0.082$), or the formulated feeds and the FrM fed lobster whole body dry matter levels ($t=-2.296$, $df=15$, $P=0.771$).Whole body crude lipid content was highest in the FrM lobsters at 1.6%, whereas the SQM contained below half that level of crude lipid at 0.64% (Table 3.3). Whole body composition of the initial animals (1.05%) was similar in crude lipid content to the CO, FOL and MM lobsters (Table 3.3).

3.4.2 Digestive gland

The crude lipid levels in the digestive gland were significantly lower in the SQM, FO, and TO fed lobsters than in the CO, FOL and MM fed lobsters which were similar in crude lipid content to the initial lobsters (10%) (Table 3.3). The FrM fed lobster digestive glands contained 12.3% crude lipid, significantly higher than the other formulated feeds ($t=-3.669$, $df=15$, $P=0.002$). Crude protein levels in the digestive gland ranged between 12.9 – 15.7% and but did not differ significantly ($t=-1.199$, $df=15$, $P=0.249$) from the composition of FrM fed lobsters (16.2%). The dry matter content of the tissues was not significantly different between the formulated feeds ($F=2.029$, $df=5,1$, $P=0.168$), however the average of the formulated feeds (24.44±0.9%) was significantly lower than the dry matter content of the FrM fed lobsters (31.29±1.44%) ($t=-2.554$, $df=15$, $P=0.022$). The fresh mussel fed lobsters were similar in whole body crude lipid composition (1.61%) to the MM fed lobsters, but significantly higher than the pooled crude lipid content of the formulated feed fed lobsters (1.06%) ($t=-2.627$, $df=15,2$ $P=0.019$).

3.4.3 Growth efficiency

Feed efficiency ratio (FER) and protein efficiency ratio (PER) (Fig. 1A) did not differ significantly between the formulated feeds (F=3.058, df=5,1, P=0.069) and (F=3.611, df=5,1, P=0.045) respectively. However, the lipid efficiency ratio (LER) (Fig. 1B) was significantly higher in the TO and FOL feeds than the SQM feed (F=4.001, df=5,1, P=0.034). When further assessing the retention of the consumed nutrient, the retention of consumed crude lipid as body lipid (PLV) (Fig. 2B) behaved differently where lipids fed in the MM and FOL were more efficiently retained than the SQM feed (F=5.768, df=5,1, P=0.012). The productive protein value (PPV) (Fig 2A) was significantly higher in the MM, FOL and TO feeds than the SQM feed (F=5.864, df=5,1, P=0.011).

3.5 Discussion

3.5.1 Growth performance

As has been observed in past growth studies with southern rock lobster, there was a marked difference in growth rate between fresh mussel fed lobsters and formulated-feed fed lobsters (Crear et al., 2002; Ward et al., 2003). The main interest for aquaculture nutrition is to reduce dependence on natural foods, and to produce commercial formulated feeds that promote superior growth. Therefore, this study was focussed on understanding the differences in growth between the formulated feeds, and investigating where potential to increase growth exists. Growth performance promoted by these formulated feeds was better than past studies with *Jasus edwardsii* of similar size fed formulated feeds of fish meal and fish oil origin (Ward et al., 2003). Although there were slight differences between the dietary lipid levels, these did not affect growth performance. The specific growth rate of the formulated feeds in a similar trial ranged between 0.5-0.8 $\%.d^{-1}$ (Ward et al., 2003), which was lower than the range 0.9-1.6 recorded in the current study. The SGR of the lobsters fed fresh mussels in Ward et al., (2003) was 1.3 compared to 2.2 in the current study. Increases in growth rates may relate to the shorter experimental period and reduced animal density in the current study. Feed (5B) from Ward et al., (2003), had the most similar chemical composition to the fish

meal/fish oil feed in the current study, yet produced a lower SGR of 0.7 (5B) compared to the present study SGR of 1.26 (FO).

3.5.2 Growth efficiency

Lipid has been suggested as the major energetic substrate in larval *J. edwardsii* is essential for growth and survival in juvenile lobster stages (Jeffs et al., 1999; Lemmens, 1994) and in other crustacean species (Chang and O'Connor, 1983). However, the potential of alternate lipid sources to improve growth rates has not been investigated in spiny lobsters. The growth expressed as PER was not significantly different among the lobsters, however the PPV was significantly higher in lobsters fed MM and lowest in the lobsters fed SQM. Poor PPV indicates a high reliance on protein catabolism to meet energetic needs, rather than deposition as tissue growth.

The LER varied considerably and lobsters fed TO and FOL gained significantly more weight for ingested lipid than lobsters fed SQM, suggesting the TO and FOL lipid profiles promoted growth. While TO and FOL prompted weight gain, the PLV indicates that the lipid in MM and FOL was retained most efficiently as lipid in the body. The reason why TO is a better fuel for growth, and MM while promoting growth, is better retained as depot lipids needs to be understood. It appears that the addition of lecithin in FOL improved the capacity of a fish oil based feed (FO) to promote both growth and more efficient storage of lipids.

The accumulation of lipid in the digestive gland during the intermoult phase has been shown to directly relate to the growth increment in *J. lalandii* (Cockcroft, 1997), therefore understanding the factors causing the differentiation in lipid retention is of interest in order to improve potential lipid accumulation in order to improve growth rates. The PER of 67.21 (FO) in the present study was similar to the PER of (5B) (79.8 ±17.81) (Ward et al., 2003). The highest PPV observed in MM, FOL and TO was significantly greater than the SQM feed. CO was the only feed that did not produce a significantly different PPV to the SQM. In the lobsters fed CO and SQM, ingested protein was catabolised for energy and not converted to growth, as indicate by low PPV.

3.5.3 Energy source

Fish oil replacement is well recognised as necessary in future aquaculture feed development (Bell, 1998), various oils have been tested in crustacean feeds with varying success. While in the present study, lobsters had greatest weight gain when fed TO, MM and FOL. Marine lipids have been reported to produce superior growth to vegetable oils in *Penaeus vannamei* (Lim et al., 1997), but a combination of marine and vegetable is better than a solitary oil from either source (D'Abramo, 1989). D'Abramo et al. (1980) used combination pairs at 4% and 2% of tuna oil, cod liver oil and corn oil, and suggested that the inclusion of marine oils significantly increased growth above the purified control feed (79F) containing 4% cod liver oil and 2% corn oil. The addition of lecithin to the fish oil feed suggested an increase in weight gain and improved PLV, however this was not significant. Lecithin has been reported to significantly increase weight gain of feeds irrespective of their main lipid source (Piedad Pascual, 1986). Weight gain of *P. monodon* fed prawn head oil, soybean lecithin and an oil blend was significantly higher than in prawns fed sardine oil, cod liver oil and an oil deficient feed, while survival was greatest in prawns fed soybean lecithin, sardine oil and cod liver oil (Ahamad Ali, 1990). Improved survival was observed when lecithin was raised from 3% to 6% lecithin, and best growth occurred where lecithin made up 46% of the total lipid content of the feed (Briggs et al., 1994).

The inclusion of soybean lecithin with fish oil in the present study tended to improve growth, but this was not confirmed statistically. The supplementation of soybean lecithin with menhaden fish oil, coconut oil, soybean oil and peanut oil statistically improved growth compared to dietary oils without lecithin (Gonzalez-Felix et al., 2002). Furthermore, the digestive gland of prawns fed menhaden fish oil as the sole lipid source contained 10.1% lipid, but when fish oil was supplemented with 3.1% soybean lecithin, lipid content of the digestive gland fell to 5.2% lipid Gonzalez-Felix et al., (2002). In the current study TO resulted in lower digestive gland lipid levels, which supports the findings of D'Abramo et al. (1980), where the addition of TO reduced the depot fat (neutral classes) in the digestive gland in *H. americanus* compared to cod liver and corn oil feeds. Furthermore, when substituting fish oil with plant oils in *P. monodon* prawn feeds, feeds containing neutral lipids derived from cod liver oil in the form of TAG and

FFA promoted better growth than methyl esters (ME) or ethyl esters (EE) (Glencross and Smith, 1997).

3.5.4 Digestive gland

The vast differences in digestive gland lipid composition, and marked changes in PPV and lipid indices between feeds in this study supports D'Abramo's (1980) observation that the quality of lipid directly affects the metabolism and storage of that lipid. High levels of lipid in the digestive gland of slow growing western rock lobsters was suggested to be linked with a pathological condition similar to the excessive lipid accumulation described in vertebrate species (Tsvetnenko et al., 2000) and has been recorded in past growth trials in *J. edwardsii* (Johnston et al., 2003; Ward et al., 2003) and *H. americanus* (D'Abramo et al, 1980). Whether this is due to inappropriate lipid levels or fatty acid profiles is unclear. High dietary lipid levels tended to increase lipid carcass content in *P. monodon* fed with cod liver oil, soybean oil and soybean lecithin (Briggs et al., 1994). The feeds in this study were based on the optimal lipid levels and nutrient ratios identified in Johnston et al., (2003) and Ward et al., (2003), and accordingly the digestive gland lipid levels were not excessive, and remained similar to (MM, CO, FOL) or below those of fresh mussel fed lobsters (TO, FO, SQM). This may indicate either that dietary lipids provided were at optimal levels for efficiently metabolism or were not of appropriate profile for energy storage. The possibility that the lipid class and fatty acid class of the lipid is driving growth differences is worthy of investigation.

3.6 Conclusion

Mollusc meals in crustacean feeds have been reported to produce optimal growth, however the disparity between squid meal and mussel meal is perplexing, and further investigation into the squid meal lipid profile and possible degradation in order to understand the massive growth decrease is underway. There appears a strong trend between the fatty acid profile or quality of the lipid and the rate at which is it metabolised or stored in the digestive gland and muscle tissues. Understanding the

impact of lipid on growth and energy storage, and the appropriate ingredient in which to supply dietary lipids will improve our capacity to refine feed performance further.

Table 3.1. Ingredient inclusion rates and chemical composition of feed formulations

Ingredient g.kg^{-1}	CO	FOL	FO	TO	SQM	MM
Fish oil		18.4	63.9		69	70.7
Canola oil	64.5					
Tuna oil				64.3		
Soy lecithin		50.8				
Fish meal	599	599	599	599		
Squid meal					556.0	
Mussel meal						649.1
Prawn meal	90	90	90	90	90	90
BO11C starch	170	170	170	170	170	170
Diatomaceous earth	51.0	46.9	50.9	0	85.0	1.0
Manucol	60	60	60	60	60	60
TSP Phosphate	20	20	20	20	20	20
Vitamin premix	2	2	2	2	2	2
Vitamin C	1	1	1	1	1	1
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5
Banox E	0.2	0.2	0.2	0.2	0.2	0.2
Cholesterol	0.1	0.1	0.1	0.1	0.1	0.1
Yttrium oxide	1	1	1	1	1	1
Ytterbium acetate	1	1	1	1	1	1
Chemical composition g.kg^{-1}						
Crude protein	404.5	414.9	402.8	401.4	400.7	365.6
Crude lipid	117.5	130.0	123.0	122.5	164.2	155.2
Gross energy	177.8	181.4	176.5	179.2	184.2	200.4
CHO	353.2	324.9	308.7	361.2	302.4	288.7
Ash	60.0	53.9	104.9	61.8	82.1	118.0
Moisture	64.8	76.3	60.6	53.1	50.6	72.5

CO = canola oil, TO=tuna oil, FO=fish oil, FOL=fish oil and lecithin blend, MM=mussel meal, SQM=squid meal, FrM= fresh mussel

The fresh mussel composition (DM basis) was crude protein 47.7%, crude lipid 8.2%, gross energy 18.05 MJ.kg^{-1}, ash 1.8%.

Table 3.2. Growth performance and nutrient retention of juvenile *J. edwardsii* fed feeds containing varying lipid sources over 10 weeks

	Units	CO	FOL	FO	TO	SQM	MM	*FrM*	*P*
Initial weight	g	1.59	1.50	1.62	1.57	1.55	1.53	1.58	0.959
S.E.		*0.04*	*0.10*	*0.10*	*0.11*	*0.20*	*0.04*	*0.13*	
Final weight	g	3.45^{ab}	4.36^{b}	3.81^{ab}	4.26^{b}	2.78^{a}	4.26^{b}	7.23	**0.018**
S.E.		*0.27*	*0.40*	*0.23*	*0.24*	*0.11*	*0.24*	*0.00*	
Weight gain[1]	g	1.86^{ab}	2.87^{b}	2.19^{ab}	2.70^{ab}	1.23^{a}	2.74^{ab}	5.65	**0.038**
S.E.		*0.31*	*0.49*	*0.33*	*0.18*	*0.31*	*0.25*	*0.13*	
Survival	%	96.67	76.67	91.11	82.22	86.67	95.56	100.00	0.565
S.E.		*3.33*	*16.67*	*5.88*	*9.69*	*6.67*	*4.44*	*0.00*	
SGR[2]	$\%.d^{-1}$	1.13	1.57	1.26	1.48	0.87	1.51	2.24	0.102
S.E.		*0.15*	*0.23*	*0.18*	*0.07*	*0.25*	*0.10*	*0.12*	
DGI[3]		5.10	5.23	4.95	5.36	5.73	5.72	4.47	0.591
S.E.		*0.55*	*0.09*	*0.17*	*0.52*	*0.22*	*0.31*	*0.36*	
Feed intake[4]	g DM	127.79	119.24	117.88	115.52	107.74	122.81	*n.d.*	0.262
S.E.		*4.35*	*1.30*	*0.90*	*8.12*	*0.92*	*4.41*	*n.d.*	
FER[5]	%	0.22	0.32	0.27	0.33	0.17	0.33	*n.d.*	0.069
S.E.		*0.03*	*0.04*	*0.04*	*0.04*	*0.04*	*0.03*	*n.d.*	

CO = canola oil, FOL=fish oil and lecithin, FO=fish oil, TO=tuna oil, MM=mussel meal, SQM=squid meal, FrM= fresh mussel

n.d. = not determined

[1]weight gain = final wt – initial wt

[2]SGR = ln(initial wt)-ln(final wt)/days*100

[3]DGI = digestive gland weight/whole body weight*100

[4]Dry weight of feed fed after adjustments for uneaten feed and leaching

[5]Feed efficiency ratio (FER) = wet weight gain/feed intake (DM)

Values that are not significantly different share a common superscript.

Table 3.3. Chemical composition of juvenile lobster whole body and digestive gland tissues as percentage of their wet weight before and after feeding differing lipid sources over 10 weeks.

	CO	FOL	FO	TO	SQM	MM	*Fr.M*[1]	Initial	*P*
Whole body									
Lipid	1.26^{bc}	1.28^{bc}	0.87^{ab}	0.76^{ab}	0.64^{a}	1.55^{c}	1.61	1.05	**0.004**
S.E.	*0.21*	*0.30*	*0.10*	*0.02*	*0.01*	*0.11*	*0.19*	*0.00*	
Crude protein	12.08^{ab}	13.50^{b}	12.53^{b}	11.98^{ab}	10.06^{a}	13.48^{b}	12.28	12.19	**0.006**
S.E.	*0.65*	*0.71*	*0.40*	*0.29*	*0.41*	*0.38*	*1.2*	*0.22*	
Dry matter	27.10	29.25	26.64	26.13	24.26	28.31	27.39	25.33	0.082
S.E.	*0.20*	*2.42*	*0.75*	*0.55*	*0.51*	*0.75*	*0.84*	*0.00*	
Digestive gland									
Crude lipid	8.30^{bc}	9.03^{bc}	3.50^{ab}	3.66^{ab}	2.24^{a}	9.89^{c}	12.33	10.00	**0.003**
S.E.	*2.16*	*2.27*	*0.28*	*0.39*	*0.20*	*1.13*	*1.48*	*0.31*	
Crude protein	12.94	15.71	15.61	14.61	15.65	14.82	16.16	19.70	0.362
S.E.	*0.93*	*0.94*	*0.24*	*1.27*	*0.01*	*0.76*	*0.01*	*0.18*	
Dry matter	22.36	27.10	23.86	22.19	22.13	28.47	31.29	23.67	0.168
S.E.	*0.15*	*1.85*	*0.96*	*2.33*	*0.33*	*2.65*	*1.44*	*0.00*	

CO = canola oil, TO=tuna oil, FO=fish oil, FOL=fish oil and lecithin blend, MM=mussel meal, SQM=squid meal, FrM= fresh mussel.

[1] Fresh mussel excluded from statistical analysis. Significant differences in response variables by row of formulated feeds by one-way ANOVA are indicated in boldface type. Values that are not significantly different share a common superscript.

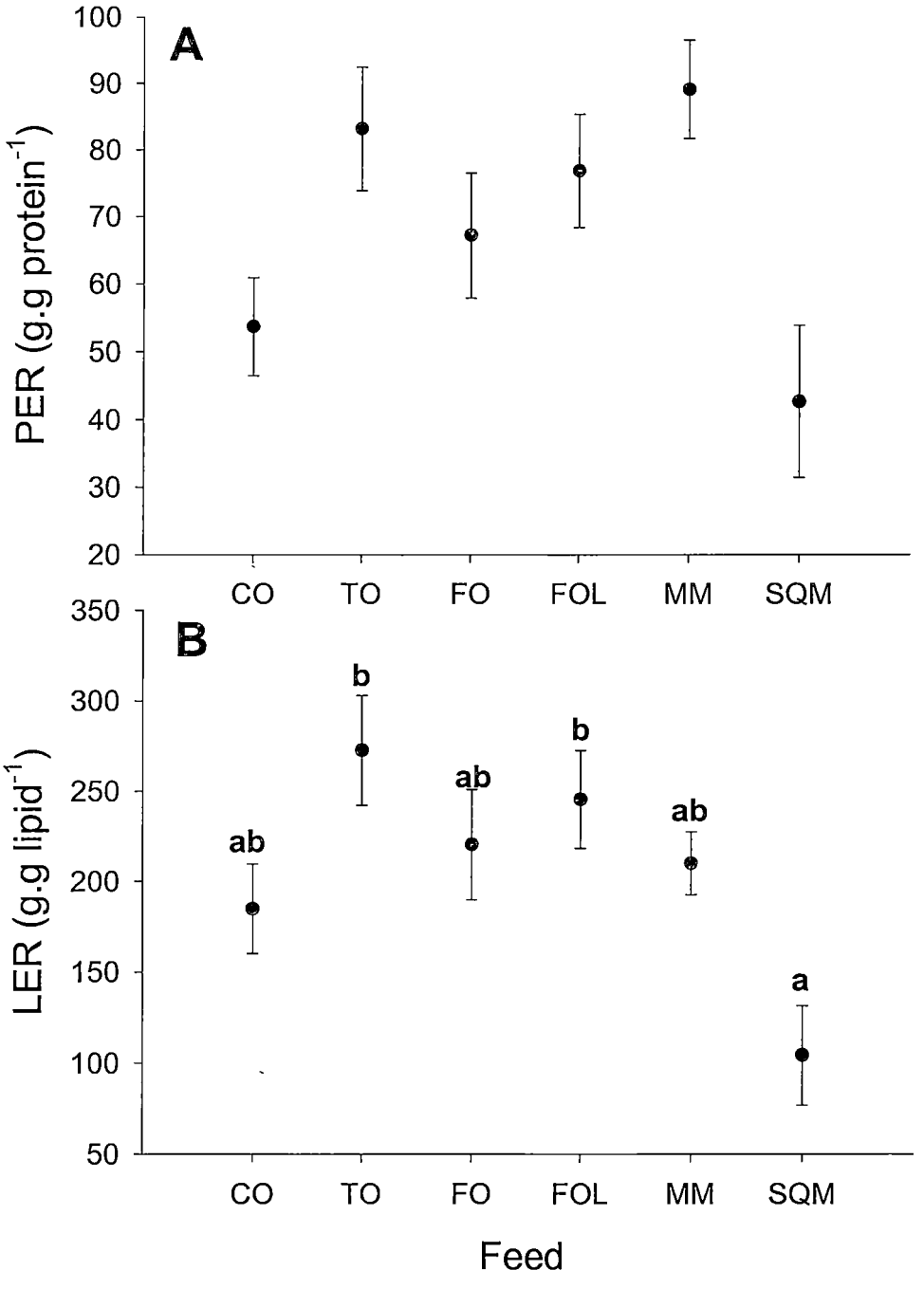

Figure 3.1. Protein efficiency ratio, PER (A) and lipid efficiency ratio, LER (B) of juvenile *J. edwardsii* fed feeds of differing lipid source over a 10 week growth trial. Values that are not significantly different share a common superscript. CO=canola oil, TO=tuna oil, FO=fish oil, FOL=fish oil and lecithin blend, MM=mussel meal, SQM=squid meal.

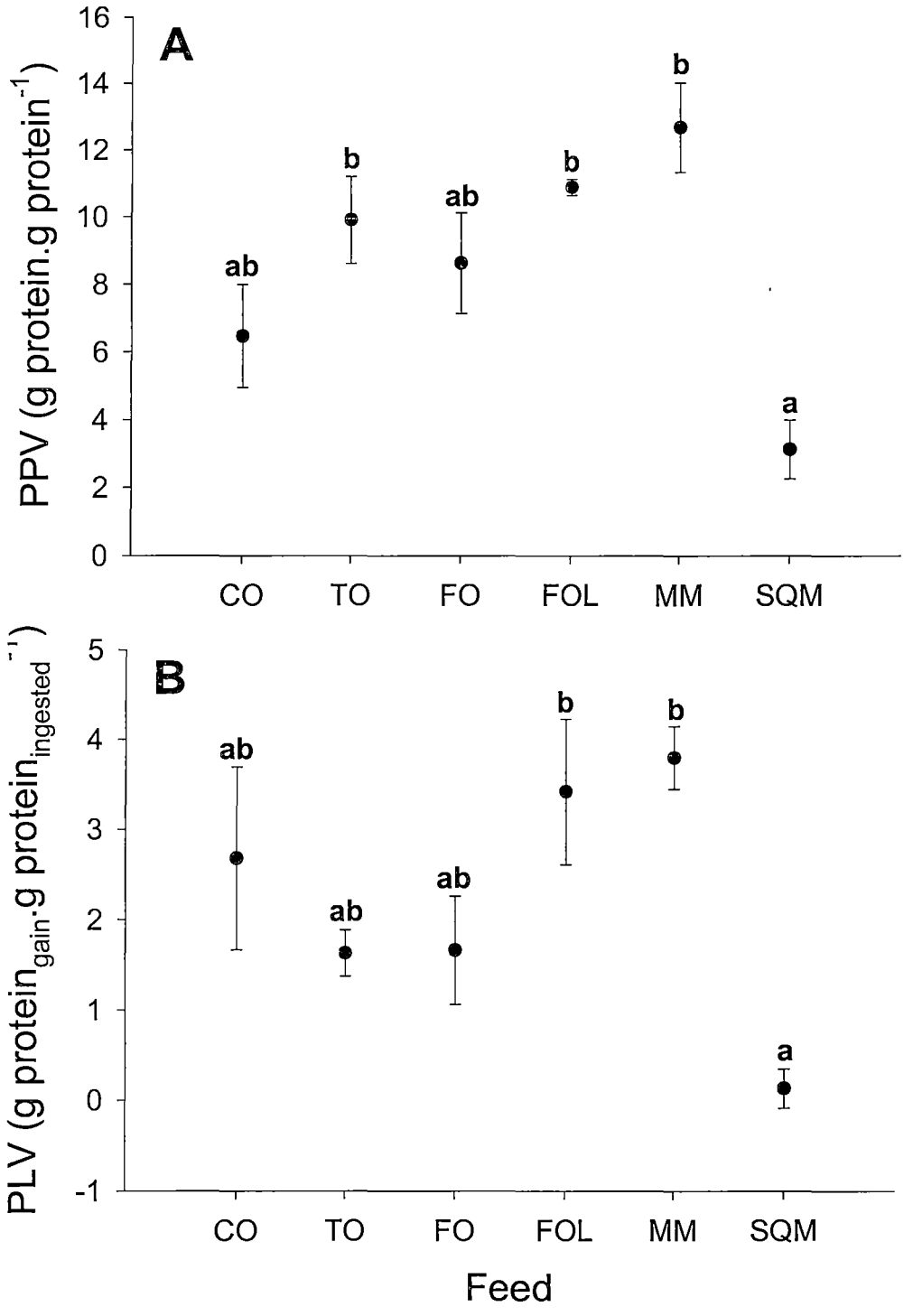

Figure 3.2. Productive protein value, PPV (A) and productive lipid value, PLV (B) of juvenile *J. edwardsii* fed feeds of differing lipid source over a 10 week growth trial. Values that are not significantly different share a common superscript. CO=canola oil, TO=tuna oil, FO=fish oil, FOL=fish oil and lecithin blend, MM=mussel meal, SQM=squid meal.

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Chapter 4

Digestive gland histology of juvenile lobster, Jasus edwardsii, in response to different dietary oil sources

4.1 Abstract

Experimental lobster feeds are currently based on fish meal and fish oil formulations, and although good survival and growth up to that of lobsters fed fresh blue mussels has been achieved, varying protein levels in feeds has not increased growth to that of natural food. This experiment assessed the performance of a range of oils and oil containing ingredients (fish oil, fish oil with added lecithin, canola oil, tuna oil, mussel meal, squid meal) in their commercially available form in lobster feeds. Performance was assessed by growth rate, survival, final chemical composition and gross nutrient retention and retention efficiency. Groups of 15 post-larval lobsters were randomly allocated one of six test feeds in triplicate. Two remaining tanks were fed freshly opened blue mussels. Lobsters were fed daily to excess, and weight measured every three weeks. At the end of the experiment final weights of whole body and digestive gland were measured. There were no significant differences in survival, weight gain or specific growth rate among the feeds. However, the squid meal fed lobsters had a significantly lower lipid efficiency ratio and lipid productivity value than for all other feeds. Digestive glands were sampled and processed for normal and resin histology for cellular structure, to illustrate the localisation of lipid, glycogen and protein. Cellular structure and cytology of the digestive gland tubule epithelium varied according to feed, most notably with excessive vacuolation of B-cells in lobsters fed the squid meal feed. Fast growing lobsters fed feeds (fresh mussel, mussel meal and fish oil with lecithin) had relatively fewer B-cell vacuoles and many R-cells containing lipid droplets. Canola oil, fish oil, tuna oil and squid meal fed lobsters had very few lipid droplets within the digestive gland. Mussel meal and fish oil with lecithin fed lobsters contained more lipid droplets in digestive gland tissue, but still less than observed in the fresh mussel fed animals. Reserve cells were observed in the digestive glands of lobsters that contained abundant lipid droplets and that showed good growth rates. Histology provided a useful assessment of the effects of formulated feeds on digestive gland performance and lipid storage.

4.2 Introduction

The performance of formulated feeds is generally assessed growth response and nutrient retention (Jobling, 1983). Additionally, the nutritional status is often described by indices of body condition (D'Abramo and Castell, 1997) and crude chemical composition, amino acid and fatty acid composition are used less often (Smith et al., 2004). However, the localised effects of feeds within body tissues are less often investigated. Consequently, more detailed examination of key organs using methods to identify cellular structure and energy storage have been recommended in conjunction with growth experiments (Vogt et al., 1986; Gropp and Tacon, 1993; D'Abramo and Castell, 1997; Johnston et al., 2003). Such examination may reveal important changes prior to gross indices, where a visible reduction in energy storage or pathological changes to the digestive gland may precede the gross signs of reduced growth, nutrient deficiency or starvation that develop over an extended period of time.

The digestive gland has several key roles and has potential to act as an indicator organ (Vogt et al., 1985). The digestive gland is the main site of digestion and energy storage in crustaceans, and in particular facilitates the emulsification, digestion, metabolism, storage and transport of lipids to the tissues to maintain physiological processes (O'Connor and Gilbert, 1968; Chang and O'Connor, 1983; Brunet et al., 1994). The gland is a symmetrical bi-lobed organ, occupying the majority of space in the carapace (Vonk, 1960). The lobes of the digestive gland consist of blind ending tubules, composed of a single layer of columnar epithelium; the comprising cells characterized as B (blister like)-, F (fibrillar)-, R (resorptive)- and E (embryonic)-cells. The role of these cells within the digestive gland has been debated, with one theory describing the differentiation of E-cells to F-cells or R-cells (Al-Mohanna and Nott, 1989). F-cells are involved in digestive enzyme excretion. Mature F-cells differentiate to B-cells and facilitate intracellular digestion within large digestive vacuoles which rupture into the lumen after digestion (Al-Mohanna and Nott, 1987; Al-Mohanna and Nott, 1989). R-cells absorb soluble nutrients from the lumen and store energy reserves, usually as glycogen and lipid droplets. The emphasis on each of these three roles fluctuates throughout both the ecdysial and digestive cycle (Travis, 1955; Brunet et al., 1994).

Furthermore, histological changes in digestive gland tissues that relate to nutritional stressors have been described in terrestrial (Storch, 1984) and aquatic decapod species (Rosemark et al., 1980; Storch et al., 1982; Papathanassiou and King, 1984; Johnston et al., 2003; McLeod et al., 2004). Differences in the digestive gland size, structure and appearance have been reported in relation to changes in feed composition and starvation (Rosemark et al., 1980; Piedad-Pascual et al., 1983; Vogt et al., 1986; Catacutan and De La Cruz, 1989; Johnston et al., 2003). Feeding affects the localization of energy reserves and adverse effects of nutrient level or intake on health and survival with respect to structural changes in tissue integrity in terrestrial isopods (Storch, 1984). Histological signs of nutritional stress in crustaceans may include changes in B-cell vacuolization, abundance of glycogen granules or lipid droplets, alterations to the ultrastructure of cells, infiltration of haemocytes in intertubular spaces, lattice-formation in the cytoplasm of tubules and total necrotic disintegration of the tubules and gland structure (Storch, 1984). It is worthy to note that differences in cellular ultrastructure may occur while chemical concentrations of protein, carbohydrate and lipids may remain constant within tissues (Vogt et al., 1986). Juvenile *Jasus edwardsii* that exhibited poor growth in previous growth experiments (Ward et al., 2003) had non-typical digestive gland appearance, variable lipid content and in some cases total necrosis of the digestive gland (Ward, unpublished data) as also reported in *Homarus americanus* fed nutrient deficient feeds (Rosemark et al., 1980).

There is increasing focus on the replacement of fish products in aquafeeds, and the development of future crustacean feeds will require the incorporation of alternative dietary lipids of non-fish oil origin (Bell, 1998). It is understood that crustaceans can incorporate essential fatty acids preferentially into tissue lipid classes (Teshima et al., 1977). However, the consequences of replacing fish oil with alternate oils in feeds on the function and cellular ultrastructure of the digestive gland are unknown. Recent studies exploring dietary effects on nutritional status of *J. edwardsii* have described histological changes in digestive gland structure (Johnston et al., 2003; McLeod et al., 2004). Digestive gland dissections at the conclusion of dietary experiments have often exhibited stark changes in appearance; texture and colour, and in some cases total

necrosis with varying feed formulations warrant further investigations of digestive gland ultrastructure and function in response to dietary manipulation (personal observation).

Fatty acid profiles of fish and crustaceans have been shown to partially adopt the essential fatty acid profile of the feed. However, crustaceans have also shown the ability to selectively incorporate fatty acids into different lipid classes (D'Abramo et al., 1980). The effects of changing dietary lipid profile on lipid incorporation and storage have not previously been examined in juvenile *J. edwardsii*; however it has been shown during starvation experiments of adult *J. edwardsii* that lipids were metabolised preferentially before proteins (McLeod et al., 2004). This study investigated the effects of replacing fish oil in lobster feeds with alternative lipid sources on digestive gland structure, tubule histology and lipid droplet deposition.

4.3 Materials and Methods

4.3.1 General methods

The animals used in this chapter are from the experiment described in Chapter 3. Detailed general experimental and sampling methods have been previously described in Chapter 3, (sections 3.3.1 to 3.3.12). Briefly, this chapter presents a qualitative description of the digestive gland histology, and semi-quantitative description of lipid droplet accumulation, B-cell, R-cell and reserve cell abundance in lobsters from the initial population, those fed the reference feed of freshly opened blue mussels (*Mytilus edulis*) (FrM) and formulated feeds (Table 3.1) containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM) and mussel meal (MM) *ad libitum* in a 10 week growth experiment. A 20-tank system was used with three tanks randomly allocated each of the formulated feeds, and the remaining two tanks were fed freshly opened blue mussels (*Mytilus edulis*).

4.3.2 Dissection and fixation

At the conclusion of the experiment, feeding ceased 24 h prior to sampling in all tanks. Lobsters from each tank were dried and weighed as above, then the five most median

sized lobsters in the intermoult stage (Turner, 1989) from each tank were killed in an ice-water slurry, and their digestive glands immediately dissected. Four of the digestive glands were bisected longitudinally, with half rapidly frozen in liquid nitrogen for proximate and fatty acid analysis and half immediately fixed for histology. Two halved digestive glands from two individual lobsters were fixed in 2.5% glutaraldehyde overnight and post-fixed in osmium tetroxide for lipid histology, and remaining two halves were fixed in Gendre's fluid for general and carbohydrate histology.

4.3.3 General histology

Digestive glands tissue sections (n=5) were blocked for each individual (n=2) per tank selected from each feed treatment (n=7) and the individual lobsters (n=6), where the tanks selected corresponded to those analysed for fatty acid and lipid class analyses (Chapters 5&6). Digestive glands were cut into small pieces 0.5 cm^2, fixed in Gendre's fixative for glycogen for 48 h, dehydrated, infiltrated and 5 random digestive tissue sections from each lobster were embedded in each block of paraffin wax (Tissuetek® II, RX-11B). Six 5 μm sections from random depths through each block were sectioned on a microtome (Microm, Heidelberg), and were adhered to the slide by incubation at 60°C until dry. Two sections were stained with standard haemotoxylin and eosin (H&E), the remaining four slides stained for glycogen (section 4.3.5). Slides were examined at either 200 or 400 X magnification using an Olympus BH-2 light microscope, fitted with a Leica DC 300F digital camera, Leica Microsystems AG CH-9435, Heerbrugg, Switzerland. Images were captured using Leica IM50 version 1.20 Release 9 2001. H&E sections were examined for general tubule and epithelial structure, necrosis or atrophy, and the presence of potential reserve cells in the interconnective tissues.

The proportion of R-cells and B-cells in each tubule from multiple random H&E sections from 6 individual lobsters was approximated, by giving a score between 1 and 3 to approximate the area of tubule occupied by B-cells, where 1=low numbers of B-cells (0-30%), 2=medium numbers of B-cells (30-70%), 3=high numbers of B-cells (70-100%). An identical score was used to assess the area of digestive gland tubule occupied by R-cells within the digestive gland tubule. The presence of reserve cells was indicated on a scale of 0-3 where 0=no reserve cells observed, 1=isolated singular reserve cells

identified, 2=small localisations of reserve cells (under 20 cells), 3=large localisations of reserve cells (more than 20 cells in large aggregations in interconnective tissues).

4.3.4 Lipid histology

The visual assessment of local lipid deposition within the digestive gland tubules was achieved using an osmium tetroxide lipid stain (Bell and Lightner, 1988). Digestive gland tissues were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.1) for 2 h at room temperature (20 °C), following 3 × 10 min washes in 0.1 M cacodylate buffer (pH 7.1). The lipid was darkened by post-fixation in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h at room temperature. Following further washes in the 0.1 M cacodylate buffer, the tissue was dehydrated and embedded in JB4 resin. Resin blocks were sectioned at 2 µm using glass knives on a microtome (Microm) and counterstained with polychrome blue. Lipid droplet density was scored using a 5 point scale where the total tubule area containing lipid droplets was give a score between 1 and 5 where, 1 = absent (less than 1%), 2 = low (under 10%) 3=medium (20-50%), 4=high (50-75%) and 5=very high (75-100%).

4.3.5 Histochemical stains

To assess glycogen presence in digestive gland cells, two random slides from each individual lobster from each tank were used (as described in 4.3.2). One slide was used as a negative control and was treated with salivary amylase, and one positive slide with distilled water, for 5 mins in a humid environment. Slides were rinsed in distilled water, dried at 60°C then were stained with periodic acid Schiff (Bancroft and Cook, 1994). A positive control of trout kidney was stained with the samples. Some sections contained unknown cells thought to be reserve cells. To aid identification, replicate slides were stained with mercuric bromophenol blue to assess the presence of protein (Chapman, 1975).

4.4 Results

4.4.1 General histology

The histology of digestive gland tubules appeared similar to previous descriptions of this tissue in *J. edwardsii* (Fig. 1), (for control see Fig. 1O & 1P). There was a general decrease in the organisation of epithelial structure in lobsters fed the formulated feeds (Fig. 1A-1J) where the number of indentations were reduced to 3-5 and the bulges were shallower, increasing the lumen volume. Digestive glands from the initial population and lobsters fed FrM were densely arranged and well organized showing approximately 5-6 indentations and bulges in the epithelium (Fig. 1M-1P). The epithelial cells comprised of the four main cell types B-cells, F-cells, R-cells and E-cells (Travis, 1955) were present, however M-cells as described by Al-Mohanna and Nott, (1989), were not seen. E-cells were predominantly found in the distal tips of tubules, while F-cells, R-cells and B-cells appeared throughout the proximal regions of the tubule.

4.4.2 B-cells

The B-cells were found mainly adjacent to the lumen of digestive gland tubules, and in most treatments were characterised by the presence of a large vacuole. The morphology of B-cells and abundance of B-cell vacuoles (Table 1) appeared to change with feed treatment. Lobsters from the formulated feeds, TO, MM, FOL, and CO contained similar sized and evenly distributed B-cells. Epithelial cells from lobsters fed FrM contained fewer B-cells than the formulated feeds. The FrM B-cell vacuoles were of regular shape and size and were predominantly situated adjacent to the lumen (Fig. 1A). In contrast, in SQM fed lobsters, and to a lesser extent FO fed lobsters, B-cells were the most abundant epithelial cell type. SQM B-cells were enlarged, often binucleated and commonly extended from the lumen to the basement membrane (Fig. 1B).

4.4.3 F-cells

F-cells were present in the digestive glands of lobsters fed all feeds (Fig. 1). In TO (Fig. 1G), MM (Fig. 1K) and FrM (Fig. 1M) the F-cells contained centrally located nuclei and were columnar, extending from the lumen to basement membrane. In SQM (Fig.1I) and to a lesser extent FO (Fig. 1E), CO (Fig. 1A), F-cells were compressed near the

basement membrane and the distal regions of the epithelium were occupied largely by B-cell vacuoles.

4.4.4 R-cells

R-cells in the FrM and initial lobsters (Fig. 1M to 1P) were columnar and regularly spaced throughout the tubule. The abundance of R-cells and density of lipid droplets within R-cells, differed with feed (Table 1). Lipid droplets were distinctly situated within R-cells, and in occasional sections within the tubule lumen (Fig. 1L). Lipid droplets were generally of similar size and in FrM densely distributed throughout the entire tubule (Fig. 1L). FOL and MM also contained moderately dense accumulation of lipid droplets (Fig.1D & 1L respectively). TO, CO, FO had very few lipid droplets which were often densely arranged in a few isolated R-cells compressed in the proximal region of the tubule (Fig. 1H, 1B & 1F respectively). SQM contained isolated singular lipid droplets within the tubule (Fig. 1J). No PAS positive glycogen granules were observed within the R-cells, regardless of feed. The control slides were PAS negative after digestion in salivary amylase (Fig. 2A & B).

4.4.5 Cells in the interconnective tissues

Intertubular spaces contained loose connective tissues, haemocytes, haemal sinuses and reserve cells in some dietary treatments (Table 1). There was no PAS positive material resembling glycogen granules within the epithelial cells, or in the interconnective tissues. However, large spherical cells with peripheral nuclei were observed in the interconnective tissues of some lobsters (Fig. 2C & 2D). The spherical cells were strongly PAS positive (Fig. 2E), however they were also PAS positive in the negative control of the same tissues (Fig. 2F). The positive control slides containing trout kidney were PAS negative after digestion in salivary amylase. The spherical cells exhibited a protein positive strong blue/red metachromasia when stained with mercuric bromophenol blue (Fig. 2G) and were similar to previous descriptions of reserve cells (Johnson, 1926; Remlath and Adiyodi, 1995) and are referred to as reserve cells in this study. F-cells, cellular membranes and nuclei were also positive for protein with strong blue staining but without metachromasia (Fig. 2G).

4.5 Discussion

4.5.1 General histology

Distribution of each cell type changes in accordance with the period of digestion time post-feeding and stage in the moult cycle and position sectioned within the tubule (Allard et al., 1988; Al-Mohanna and Nott, 1989). Accordingly, only intermoult lobsters were selected for histology and all lobsters were fasted for 24 h prior to sampling to reduce variation due to feeding time. The complete digestive cycle takes at least 24 h in prawns to complete both ingestion, primary mastication and primary digestion in the stomach to take place, before secondary digestion in the digestive gland (Al-Mohanna and Nott, 1987). Therefore the present study presents processes involved with secondary digestion and the presence of chyme and partially digested materials in B-cell vacuoles confirms this was the case. The structure of digestive gland tubules was similar to previous descriptions for juvenile *Jasus edwardsii* (Johnston et al., 2003; McLeod et al., 2004). Lobsters in the present study that were fed the formulated feeds tended to show less organized epithelial cells, where the number of epithelial indentations and depth of epithelial bulges by Vogt et al. (1990) in *Penaeus monodon* fed feeds containing antinutritional factor (mimosine). A reduction in the height of epithelial cells (similar to depth of bulges) was also reported in starved *Daphnia magna* (Elendt and Storch, 1990).

Feed composition is known to affect the cellular structure of crustacean digestive glands (Rosemark et al., 1980; Storch, 1984; Vogt et al., 1985). Rosemark et al. (1980) fed suboptimal feeds to *H. americanus* and reported subsequent reductions in lipid vacuoles in R-cells, cytolysis of B-cells and necrosis of the tubule. In contrast, in the present study, the feed producing the poorest growth response was characterized by elevated numbers of B-cells compared to the reference and control lobsters. All feeds were formulated to meet the known and predicted requirements of *J. edwardsii* (Ward et al., 2003), and differed in the source of dietary oil. Therefore the proliferation of B-cells may be attributed to the ingredients used, rather than to suboptimal nutrition. Vogt et al., (1990) showed *P. monodon* fed feeds with mimosine caused a reduction in lipid droplet abundance in R-cells. The high relative proportion of B-cells to R-cells in the present study suggests SQM causes a change in the balance of activity of the digestive gland

with an increased priority on digestive processes with greatly reduced capacity for energy storage for the SQM diet. Al-Mohanna and Nott (1987) described the pre-moult stages D_2 to D_4 as a period of rapid lipid mobilisation from the R-cells in preparation for moult. Nutritionally deprived crustaceans have been shown in growth trials to cease moulting and remain at stage D_4 indefinitely (Anger et al., 1985; Al-Mohanna and Nott, 1987). Considering the poor growth performance of SQM (Chapter 3), this may partly explain the relatively low numbers of R-cells.

In contrast FrM may present a balance where digestion and absorption of nutrients is sufficient to allow prolific lipid storage for growth and physiological requirements (e.g. moulting, biosynthesis etc.) The two roles of effective digestion and sufficient energy storage are intrinsically linked and essential for growth in crustaceans. Whether more efficient digestion reflects optimal enzyme and digestive activity, or alternatively digestion of a more nutritionally balanced feed, is unclear. In all diets, F-cells were present suggesting that enzyme release and extracellular digestion of all feeds was possible. However, in the SQM fed lobsters, the F-cells were proximally compressed against the basement membrane with little or no direct contact with the lumen to secrete digestive enzymes (Al-Mohanna and Nott, 1989). In FrM lobsters, the F-cells were columnar, extending from the basement membrane to the lumen. The feeds producing mid-range growth (TO, CO, FO, FOL) had similar relative proportions of B-cells to R-cells.

4.5.2 Feed composition affects energy accumulation

The prevalence of lipid droplets in the R-cells of digestive gland tubules supported the biochemically measured lipid retention values observed in Chapter 3 where the PLV (productive lipid value) of FOL and MM diets significantly exceeded that of SQM. Lipid droplets were present in digestive glands of lobsters fed the formulated feeds, but at reduced densities to those of the FrM lobsters. Of the formulated feeds, lipid droplets densities were highest in the FOL and MM diets. There was a marked increase in lipid droplets in the FOL as compared to the FO feed, and provides visual evidence to support increased lipid mobilisation and deposition with the inclusion of a phospholipids in feeds. Low digestive gland lipid levels in *H. americanus* have been biochemically

measured for tuna oil and fish oil based feeds (D'Abramo et al., 1980), and was suggested in part due to the highly unsaturated nature of the oils which were not used for neutral lipid depot fats. This may provide explanation for the lack of substantial lipid droplet formation in the FO and TO fed lobsters. Lipid droplets are rapidly lost from R-cell tissues in starved *J. edwardsii* (McLeod et al., 2004) and *D. magna* (Elendt and Storch, 1990), and lipid has been shown to be the major energetic substrate for puerulus of *J. edwardsii* (Jeffs et al., 2002).

The lack of glycogen granules has been described previously in stage C intermoult lobsters, and was explained by the rapid utilization during this stage for the synthesis of new tissues (Travis, 1955). While this supports the lack of glycogen observed in adult *J. edwardsii* (McLeod et al., 2004), juvenile *Homarus americanus* (Anger et al., 1985), *Penaeus monodon* (Vogt et al., 1985) and *P. vannamei* (Caceci et al., 1988), it is in contrast to a previous study with juvenile *J. edwardsii* (Johnston et al., 2003), which cannot be explained. Spherical cells, presumed to be reserve cells as described by (Travis, 1955) were present in the connective tissues of some dietary treatments.

4.5.3 Potential relationship between feed, reserve cell accumulation and growth

The presence of reserve cells in the best performing diets is of particular interest when considering the capacity of feeds to promote energy storage, and the apparent lack of glycogen storage. Reserve cells have been reported in many crustacean species including blue crab, *Callinectes sapidus* (Johnson, 1926), blue shrimp *Penaeus stylirostris* (Bell and Lightner, 1988), Moreton bay bug, *Thenus orientalis* (Johnston, 1996) and the Caribbean spiny lobster, *Panulirus argus* (Travis, 1955) and can be found in almost all the connective and integumental tissues of wild *J. edwardsii* (S. Pyecroft, personal communication). The role of reserve cells is unclear and has been linked with lipid, carbohydrate, protein and calcium storage (Johnson, 1926; Travis, 1955; Remlath and Adiyodi, 1995). The absence of glycogen granules in the R-cell cytoplasm is difficult to explain. The strong PAS positive response of reserve cells may indicate an alternative site of glycogen storage in *J. edwardsii*. Johnson (1926) noted the presence of abundant reserve cells in good condition animals, and the depletion of reserve cells during starvation or disease. Johnston (1996) suggested the role of reserve cells as energy

storage associated with *T. orientalis* in good nutritional status, and suggested their composition as an undescribed complex containing glycogen and protein, which supports the description by Johnson (1926), and that of the present study. Glycogen may have been absent from the digestive gland epithelium due to preferential use for energy, or alternatively glycogen may have been combined into a glycoprotein complex within the reserve cells. Lobsters under stress of disease or infection have a marked reduction or absence of reserve cells, especially in areas of tissue surrounding lesions (S. Pyecroft, personal communication). In the present study, lobsters under severe nutritional stress that did not grow, contained no reserve cells. The factor(s) governing the deposition of reserve cells is unknown, however the cells are known to have varying roles and chemical composition throughout the ecdysial cycle (Travis, 1955). As an energy rich reserve absent in stressed animals, it may form an intermediate or long term lipid and glycogen reserve in addition to energy reserves within R-cells in the tubules. Abundance of reserve cells in the fastest growing FrM fed lobsters, and considerable numbers in MM and FOL feeds may indicate potential relationships between lipid retention, mobilisation and reserve cell abundance. The differences in dietary oil source caused visually significant changes in the patterns of energy storage in the digestive gland of lobsters. It appears that the addition of phospholipid rich oils in feeds may play a role in the effective formation of lipid droplets in the digestive gland.

The inherent variation of epithelial cell distribution warrants caution in interpreting relative cell abundances. However, the consistent epithelial cell response to feeds, and distinct patterns in lipid deposition quantified by chemical measurement of lipid, support the use of histology in visualising epithelial changes in response to feed. Quantifying changes in the abundance epithelial cells may require more replication of individual lobsters, and measurement of the feeding history of individual lobsters, which was beyond the scope of the present study. However, the qualitative and semi-quantitative data presented suggests potential to use histology to describe nutritional effects of feed formulations in *J. edwardsii*. The importance of identifying ingredients that promote the storage of energy reserves, and the relative importance of lipid droplet deposition and reserve cells for fast growth, deserves further research.

Table 4.1. Semi-quantitative assessment of the total area within each digestive gland tubule (%) that contains lipid droplets, B-cells or R-cells and the presence of reserve cells in juvenile lobster *Jasus edwardsii* from an initial population, fed a reference feed of fresh blue mussels *Mytilus edulis* (FRM) and a range of formulated feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) in a 10 week growth experiment.

Feed	Fig. No	Lipid score[1]	Fig. No	B-cell score[2]	R-cell score[3]	Reserve cells[4]
CO	1B	1	1A	2	1	0
FOL	1D	4	1C	2	2	3
FO	1F	2	1E	2	1	1
TO	1H	2	1G	2	1	1
SQM	1J	1	1I	3	1	0
MM	1L	3	1K	2	2	2
FrM	1N	5	1M	1	3	3
Initial	1P	3	1O	2	2	1

[1] Lipid score = tubule area containing lipid droplets was scored from 1 to 5 where, 1 = absent (less than 1%), 2 = low (under 10%) 3=medium (20-50%), 4=high (50-75%), 5=high (75-100%).

[2] B-score (scale 1-5) area of tubule containing B-cells where 1=low numbers of B-cells (0-20%), 2=medium numbers of B-cells (20-80%), 3=high numbers of B-cells (80-100%)

[3] R-score (scale 1-5) area of tubule containing R-cells where 1=low numbers of R-cells (0-20%), 2=medium numbers of R-cells (20-80%), 3=high numbers of R-cells (80-100%)

[4] Reserve cell score (scale 0-3) where 0=no reserve cells observed, 1=isolated singular reserve cells, 2=small localisations of reserve cells (under 20 cells), 3=large localisations of reserve cells (more than 20 cells in large aggregations in interconnective tissues).

Fig. 4.1 (A-H). Digestive gland histology of tubules from lobsters fed feeds containing alternate oil sources. Figs are light micrographs of digestive gland tubule cross sections stained with haemotoxylin and eosin (H&E) or post-fixed in osmium tetroxide (OsT) to identify lipid droplets. Abbreviations are B (B-cell), F(F-cell), Hc (haemocytes), Hs (haemolymph space) Ld (lipid droplet), Lu (lumen), Nu (nucleus), R(R-cell). Fig. 1. Histopathology of lobster digestive gland tubules fed squid meal (SQM), mussel meal (MM), tuna oil (TO), FrM (fresh mussel), canola oil (CO), fish oil (FO) and fish oil with lecithin (FOL) for 10 weeks. Figs. 1A-1H (1A) CO fed lobster digestive glands with medium number of B-cells, H&E. (1B) Few lipid droplets were obvious, with lessening of lumen indentation apparent, OsT. (1C) FOL fed lobsters where again the indentation and bulging of the lumen margin is reduced, and irregularly shaped B-cell vacuoles are present, H&E (1D) FOL tubules showing moderate deposition of small lipid droplets through the epithelium, OsT. (1E) FO fed lobsters have moderate B-cell abundance H&E, while the same lobsters (1F) show low deposition of lipid droplets, OsT. (1G) TO fed lobsters showed some loss of indentation and bulging, and many B-cells vacuoles in the apical cytoplasm, H&E. (1H) indicates low levels of lipid deposition in R-cells, OsT. Scale = 100 μm.

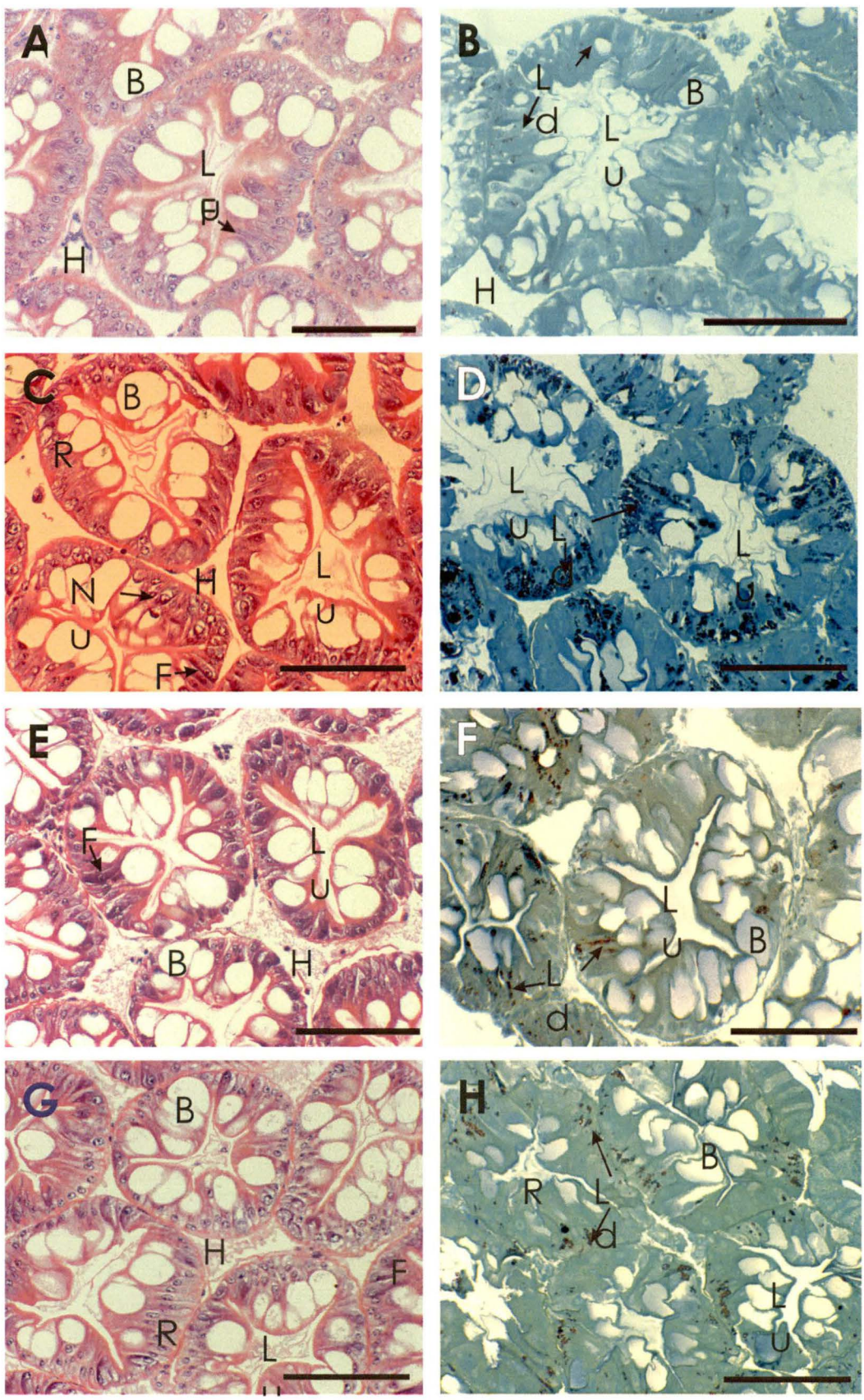
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Fig. 4.1(continued). Digestive gland histology of tubules from lobsters fed feeds containing alternate oil sources. Figs. are light micrographs of digestive gland tubule cross sections stained with haemotoxylin and eosin (H&E) or post-fixed in osmium tetroxide (OsT) to identify lipid droplets. Abbreviations are B (B-cell), F (F-cell), Hc (haemocytes), Hs (haemolymph space) Ld (lipid droplet), Lu (lumen), Nu (nucleus), R (R-cell). Histopathology of lobster digestive gland tubules fed squid meal (SQM), mussel meal (MM), tuna oil (TO), FrM (fresh mussel), canola oil (CO), fish oil (FO) and fish oil with lecithin (FOL) for 10 weeks. Fig. (1A) Digestive gland tubules of lobsters fed SQM indicating prolific B-cells, H&E and (1J) pathologically altered tubule with sparse lipid droplets in R-cells showing abundance of B-cell vacuoles, OsT. (1K) MM fed lobster tubules with characteristic epithelium organisation showing indentations and bulges, and medium abundance of B-cells, H&E. (1L) MM fed lobsters with medium level lipid storage throughout tubule, OsT. (1M) FrM fed lobster digestive gland tubules with extensive empty small lipid vacuoles and few large B-cell vacuoles, H&E. (1N) FrM shows extensive vacuolisation of lipid droplets, and epithelial organisation showing indentations and bulges, OsT. (1O) Initial lobster digestive gland tubules with large B-cell vacuoles, H&E, and (P) medium abundance of predominantly large lipid droplets throughout tubule. Scale = 100 μm.

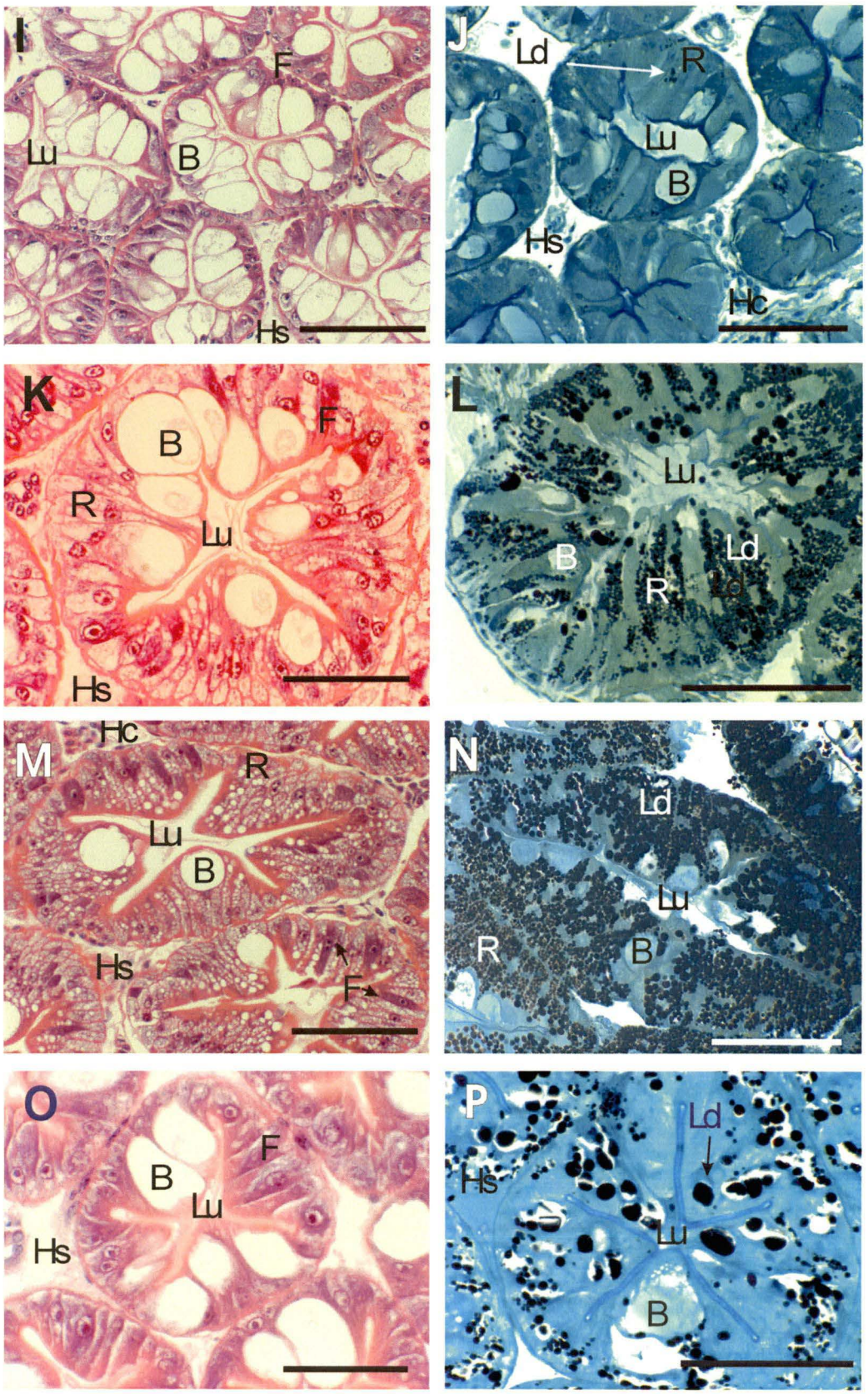
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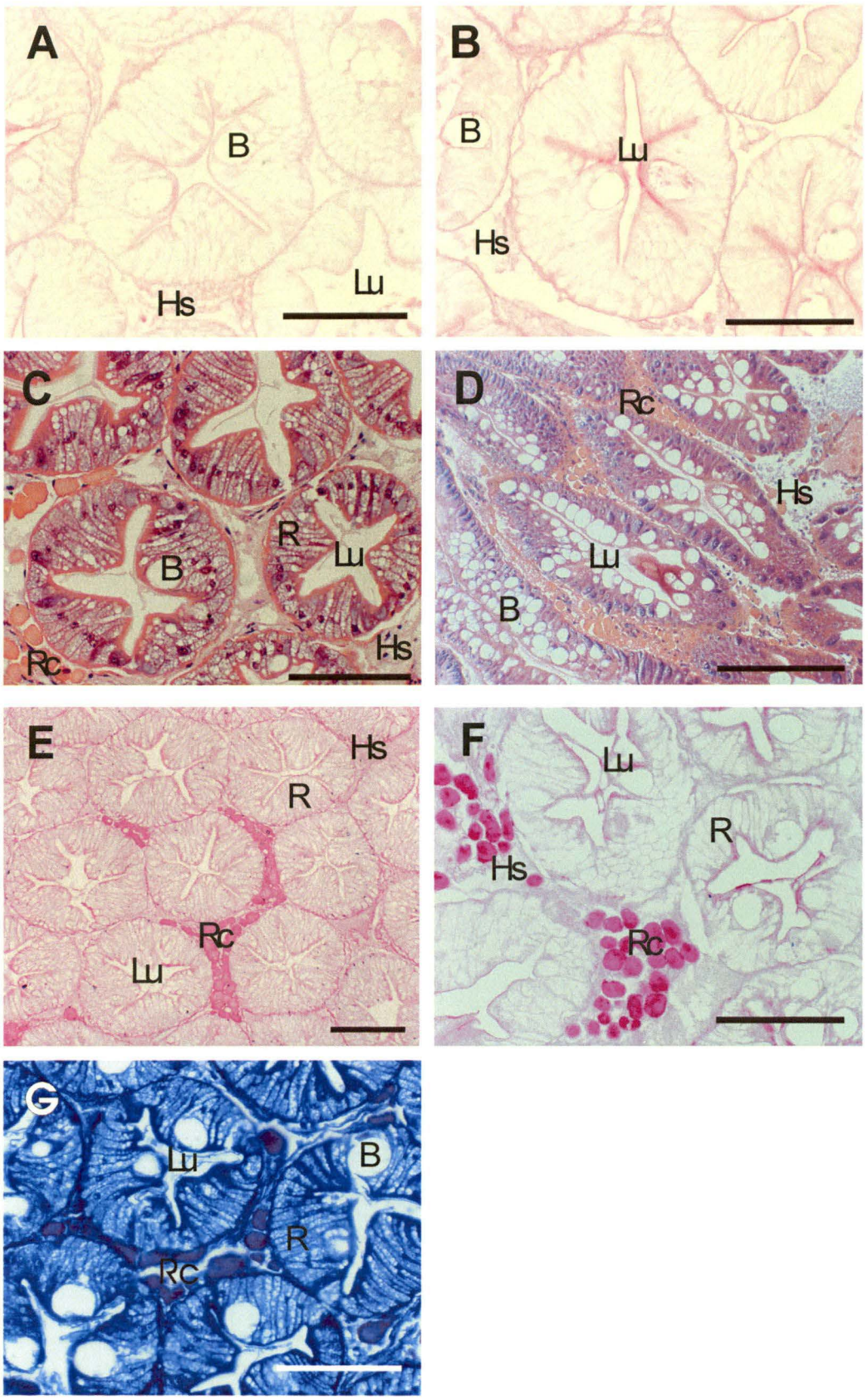
Fig. 4.2. Histochemical staining for glycogen and to identify reserve cells within digestive gland tubules from lobsters *Jasus edwardsii* fed feeds containing mussel meal (MM) and FrM (fresh mussel) after a 10 week growth experiment. Figs. are light micrographs of digestive gland tubule cross sections stained with haemotoxylin and eosin (H&E), Periodic acid-schiff (PAS +ve), negative for periodic acid Schiff where slides were treated with salivary amylase (PAS -ve) or mercuric bromophenol blue (MBB) for protein. Abbreviations are B (B-cell), Hs (haemolymph space), Lu (lumen), R (R-cell), Rc (reserve cell). Figs (2A-2G). (2A) digestive gland tubules from a lobster fed FrM indicating no positive PAS response for glycogen within the epithelium or the Hs, PAS+ve. (2B) parallel negative control section from FrM which confirmed lack of PAS positive material in 2A, PAS –ve. (2C) section of FOL fed lobster digestive gland tubule indicating presence of small localisations of Rc, H&E. (2D) oblique transverse section through digestive gland tubules of lobsters fed MM showing large aggregations of Rc in the Hs, H&E. (2E) FOL digestive gland tubules showing strong PAS positive response by the Rc, PAS+ve. (2F) Similar section of FOL fed lobster where the PAS positive response remains after treatment with salivary amylase, PAS-ve. (2G) FOL fed lobster digestive gland showing strong metachromasia (blue/red) within Rc, and strong protein response (blue) of F-cells and cellular membranes, MBB. Scale = 100 μm.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Chapter 5

Tissue lipid class composition and growth in response to different oil sources in rock lobster feeds

5.1 Abstract

Experimental lobster feeds are currently based on fish meal and fish oil formulations. Although good survival and growth of lobsters fed fresh blue mussels has been achieved, varying protein levels in feeds has not increased growth to that of natural food. This experiment assessed the performance of a range of oils and oil containing ingredients (fish oil, fish oil with added lecithin, canola oil, tuna oil, mussel meal, squid meal) in their commercially available form in lobster feeds. Groups of 15 post-larval lobsters were randomly allocated one of six test feeds in triplicate. Two remaining tanks were fed freshly opened blue mussels. Lobsters were fed daily to excess, and their weight measured every three weeks. At the end of the experiment final weights of whole body and digestive gland were measured. Performance was assessed by growth rate, chemical composition, nutrient retention and nutrient efficiency, and was related to the feed and lobster tissue composition using principal components analysis. Lipid class composition of digestive glands varied with dietary lipids. Dietary phospholipid was correlated with fast growth. High digestive gland triglyceride and diglyceride levels were correlated with high productive protein values and lipid retention in body tissues. Lobster that grew slowly contained high proportions of phospholipid and sterols. Improving the phospholipid content of formulated lobster feeds will be important to increase digestive gland triglyceride deposition, and subsequent lobster growth rates.

5.2 Introduction

Crustaceans require sources of dietary essential fatty acids (EFA), and various classes of lipids including sterols (ST) and phospholipids (PL) and carotenoids (Gonzalez-Felix et al., 2002). PL are well recognised to be of particular importance as a source of phosphatidylcholine (PC) and phosphatidylinositol (PI) which are implicated in the emulsification and transport of triglycerides (TAG) and cholesterol in the body (D'Abramo et al., 1980; D'Abramo et al., 1982; Teshima, 1997). There is evidence that the essential fatty acid (EFA) arachidonic acid (ARA) is exclusively positioned within PI (Bell and Sargent, 2003), and DHA and EPA are also preferentially conserved within PL. The non-polar sterols (ST), and the PL glycerophospholipids and sphingolipids can make up half the mass of biological membranes (Lehniger et al., 1993). ST perform roles in other important cellular functions as precursors for steroid and moulting hormones and bile acids. Diglycerides (DG) act as intracellular messengers to activate enzymes in mammals and DG with free fatty acids (FFA) have been associated with lipid biosynthesis in *P. esculentus* digestive glands (Chandumpai et al., 1991). Wax esters (WE) and hydrocarbons (HC) are found in smaller quantities in decapod crustacean tissues, although they occur in high quantities in copepods (Benson and Lee, 1975; Hakanson, 1984). Smaller quantities of other lipids are crucial as enzyme cofactors, electron carriers, emulsifying agents and hormone precursors.

The spiny lobster *Panulirus japonicus* is able to biosynthesise PL from FFA such as EPA and DHA (Kanazawa and Koshio, 1994) and crustaceans can also biosynthesise TAG from PL, but at limited rates (Kanazawa and Koshio, 1994). It is thought that the high demand for PL cannot be met by biosynthesis alone, and juvenile crustaceans require dietary PL sources to meet these requirements. The requirement for PL is poorly understood, and has been associated with cholesterol transport (D'Abramo et al., 1980; Kanazawa and Koshio, 1994).

The neutral lipids generally associated with energy storage include TAG and WE and isoprenoid fat soluble vitamins. Lipid storage in many fish species is usually as TAG which may accumulate in adipocytes surrounding the somatic tissues, and liver. In larval

J. edwardsii, TAG have not been measured until the juvenile stages (Jeffs et al., 2002). During the larval puerulus stage, fat-bodies composed of acidic lipids surround the intermediate lobes of the digestive gland in the haemocoel (Takahashi et al., 1994) and are assumed to fuel the energy demanding non-feeding on-shore migration and metamorphosis to post-larvae. No such fat-bodies have been observed in juvenile stages of *J. edwardsii*, where instead lipid reserves are composed of neutral lipids (Takahashi et al., 1994). TAG accumulation was reported in juvenile *Homarus americanus* with juvenile development, and decreases in TAG were noted at times of energetic demand surrounding ecdysis (Fraser, 1989). It is assumed that TAG form the majority of the neutral lipid that accumulates within lipid droplets in the R-cells of the digestive gland. Biochemical studies of the on-shore migration of *J. edwardsii* puerulus indicate that lipid is the energy reserve most rapidly utilised through the inshore migration of early stage lobsters (Jeffs et al., 1999). Supporting this, McCleod et al. (2004) have associated marked lipid decreases with starvation in *J. edwardsii* and with sub-optimal protein:carbohydrate:lipid ratios (Johnston et al., 2003).

Experimental crustacean feeds have included mainly oils of marine origin, where lipids are usually contributed both from a crude or refined oil (usually herring/menhaden oil) and from the residual oil within the meal. However, with recent research focussing on the replacement of fish oil in aquafeeds (Bell, 1998), and the demonstrated beneficial effects of including soybean phospholipids in juvenile crustacean feeds (Kanazawa and Koshio, 1994; Kontara, 1997), plant oils will increasingly be used in formulated crustacean feeds. Long term growth experiments have generally shown that growth is fastest when feeds contain marine oils, however, the partial substitution of fish oil with plant oils does not affect growth rates or survival in *Penaeus monodon* (Merican and Shim, 1994) provided essential fatty acid requirements are met. Therefore, there appears potential for crustaceans to utilise non-marine lipid sources.

The need to develop future aquaculture with reduced reliance on fish oil and fish meal ingredients is well recognised (Bell, 1998). To investigate the impact of ingredient substitution in commercially relevant formulated feeds, this study encompasses both a range of lipid sources (purified oils, crude oils and oil-containing meals) of both vegetable and marine origin, and assesses the effects of feed on growth and lipid class

composition of juvenile lobster tissues. The use of principal components analysis describes patterns in lipid class composition in feeds and tissues, and allows direct comparison of these patterns to the growth performance of the lobster. Through understanding the implications of changing dietary lipid class composition on the growth performance, nutrient retention, lipid composition and digestive gland condition, feeds to optimise lipid delivery and in the future, to replace fish oil use may be designed and improved over time.

5.3 Materials and Methods

5.3.1 General methods

Detailed general experimental and sampling methods are previously described in Chapter 3 (sections 3.3.1 to 3.3.12). This chapter focuses on the percentage and quantitative lipid class analysis of feeds, digestive gland and muscle tissue and relationships between lipid class composition and growth. The animals used in this chapter are from the same experiment described in Chapter 3. A 10-week growth trial was conducted to assess the effects on growth and lipid tissue compositions of feeding juvenile lobsters *J. edwardsii* with 6 formulated feeds containing alternate lipid sources *ad libitum*. A 20-tank system was used with three tanks randomly allocated each of the formulated feeds, and the remaining two tanks were fed freshly opened blue mussels (*Mytilus edulis*).

5.3.2 Tissue sampling and dissection

At the conclusion of the trial all lobsters were dried and weighed as above, then the five most median sized lobsters in the intermoult stage (Turner, 1989) from each tank were killed in an ice-water slurry, and their digestive glands were immediately removed. The hindgut and stomach were removed from the digestive gland. Four of the digestive glands were bisected longitudinally; half was immediately frozen in liquid nitrogen for biochemical and fatty acid analysis and half fixed for histology (Chapter 4). The remaining whole digestive gland was rapidly frozen in liquid nitrogen and halved for

proximate and fatty acid analysis. The remaining whole body tissues (predominantly abdominal, leg muscle tissues and exoskeleton) without the digestive gland was frozen in liquid nitrogen and is referred to as muscle tissue, (MT) throughout this chapter. References to whole body tissues (WB) in the case of initial lobsters refer to analyses of the whole body, and for all other lobsters pertain to the calculated biochemical composition using the relative proportional compositions of MT and DG. The three muscle tissues selected for biochemical analysis included a sample from the initial population of lobsters, and the lobsters fed formulated feeds with the highest growth rates (MM), the lowest growth rates (SQM). Two MT samples of 5 pooled lobsters were analysed in duplicate. Prior to chemical analyses, all tissues were freeze dried and homogenized using a mortar and pestle.

5.3.3 Lipid analysis

Total lipid was determined gravimetrically using a modified Bligh and Dyer (1959) one-phase methanol:choloroform:water (2:1:0.8, by vol) extraction for 24 h, after which the phases were separated by the addition of chloroform and water (final solvent ratio, 1:1:0.9, methanol:chloroform:water by vol). The lipid was concentrated by rotary evaporation at 40˚C. Lipid class analyses were performed immediately; samples were stored for no more than 3 days in a known volume of chloroform.

The abundance of lipid classes from a sample of total lipid was analysed using a thin-layer chromatography-flame-ionisation detector (TLC-FID) (Iatroscan MK V TH10, Iatron Laboratories, Japan). Aliquots of lipid were applied to duplicate silica gel SIII chromarods (5 μm particle size) using disposable 1μl micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper, containing a hexane-diethyl ether-acetic acid (60:17:0.2) solvent system, to separate the non-polar compounds such as WE, TAG, FFA and ST, with PL remaining at the origin. A second non-polar solvent system of hexane-diethyl ether (96:4) was used to separate hydrocarbon from WE and TAG from diglyceryl ether (DAGE). Chromarods were oven dried at 100°C and analysed immediately. The FID was calibrated for each lipid class (phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, WE (derived from fish oil), TAG (derived from fish oil) and DAGE (purified from shark liver oil);

0.1-10μg range), and the peaks were identified using DAPA software (Kalamunda, Western Australia). The total percentage of each lipid class was then calculated.

5.3.4 Statistical analysis

The normality and homogeneity of data was explored by examining residual plots. Non-normal data were transformed using arcsine transformation for percentage data, and square root or $\log_{10}$ transformation for non-percentage data. Where transformation did not improve inequality of variance, data were left untransformed. One-way analysis of variance was used to determine significance at $\alpha = 0.05$, unless otherwise specified. Tukey's HSD was used to determine significant differences between groups. Lobsters in three adjacent tanks fed SQM, FOL and TO were affected by a *Vibrio harveyii* infection in the last week of the experiment, which caused the death of smaller lobsters in these tanks. Data for these tanks were omitted from all statistical analyses and subsequent discussion.

Principal components analysis was used to describe patterns of lipid composition among lobsters fed a reference and formulated feeds, for digestive gland, muscle tissue and feed on a **percentage** and mg lipid class per animal (**quantitative**) basis. In each analysis, the lipid class components used for interpretation was determined by the magnitude of their components scores, a scree test (Cattell, 1966), eigenvalues >1 rule (Kim and Mueller, 1978), running a parallel analysis (Montaneli and Humphreys, 1976) and interpretability of the factors (Zwick and Velicer, 1986). Lipid classes with components of 0.4 were considered to contribute to the pattern of lobster lipid composition. Loadings may be accepted as low as ± 0.32 in some cases (Tabachnick and Fidell, 1996). Principal components were extracted using varimax rotation to maximise interpretability of the components. The scores of each individual animal in the PCA space were then correlated against growth and biochemical composition parameters. Correlations were tested at 0.006, to control the experiment-wise error rate of 0.05 for multiple tests using the Bonferroni method (Sokal and Rohlf, 1995).

5.4 Results

The individual lipid class content, and patterns in lipid class composition for the feeds and lobster tissues are presented in each section. Feed and tissue of lipid class content were compared between each feed using one-way ANOVA. Patterns in lipid class composition, and their relative strength in describing the variation within the data, are described using principal components. The patterns in lipid class compositions were correlated to significant growth performance parameters identified in Chapter 3.

5.4.1 Feed - percentage and quantitative lipid class composition

The feed lipid class composition differed significantly both on a quantitative and percentage basis (Table 5.1, 5.2). The crude lipid was formulated to an isolipidic level; however variation in meal oil content caused slight variation between feeds (Chapter 3, Table 3.1). While total intake of lipid per lobster differed over the experiment (0.9 to 1.3 g) ($F_{5,9}$=14.0, P=0.001), low growth in SQM was not related to high lipid intake as there was no difference in lipid intake between the lobsters fed SQM and MM (1.16 g lipid DM.lobster^{-1}) and FOL (1.3 g lipid DM.lobster^{-1}). As there were slight differences in lipid content, both quantitative and percentage composition were compared. The major variation (66.1%, PCA1) in lipid composition of the formulated feeds was attributed to high WE (2%), and FFA (17%) in SQM and similar levels in the other feeds ($F_{6,7}$=14.3, P=0.001) (Fig. 5.1A). PCA2 (28.5% total variation) described the separation of MM with high ST (2.5%), which was significantly higher than SQM, CO, FOL and FO (1.1-1.7%) ($F_{6,7}$= 12.5, P=0.002) (Fig. 5.1A). TAG content was highest in CO, FOL, FO and TO (66 to 69%), lower in MM (52%) and lowest in FrM (0.3%) ($F_{6,7}$=710.3, P<0.001) (Fig. 5.2A). FFA were significantly higher in SQM (17%) and similar in all other feeds (2-4%). DG were also highest in the SQM feed (2%) and absent in the FrM feed ($F_{6\ 7}$=85.1, P<0.001). FrM had higher PL by proportion than all other feeds (93.4%), of which SQM contained the least (17%) ($F_{6,7}$=880.3, P<0.001) (Table 5.1).

There were a strong correlations between feed lipid class composition and lobster performance (from Chapter 3, see Table 5.3) when feed PCA scores were plotted in the new two-dimensional PCA space. Weight gain was positively correlated with individual

feed PCA1 scores, where feed contained higher levels of WE, FFA, DG and low ST levels (R_{10}=0.850, P<0.001) (Table 5.4). The feed PCA1 scores were also correlated with a reduction in LER (R_{10}=-0.739, P=0.003) (Table 5.4). The feed PCA2 was negatively correlated with productive protein value (PPV) (R_{10}=-0.761, P=0.005,) and whole body crude protein levels (R_{10}=-0.743, P=0.002) (Table 5.4) which was driven largely by two data points (SQM) with low growth and low content of TAG and high PL.

When the lipid class in feeds was assessed per g DM of feed, similar results were observed where the higher WE and FFA content of SQM feeds (PCA1) explained 61% of the variation in lipid composition (Fig. 5.3A). Quantities of TAG were highest in SQM (100 mg.g DM^{-1}) and lowest in FrM (0.2 mg.g DM^{-1}) ($F_{6,7}$=698.7, P<0.001) (Table 5.2). ST was significantly higher in MM (3.9 mg.g DM^{-1}) than all other feeds ($F_{6,7}$=16.9, P=0.001), and was explained by PCA3 (17% of the total variation, not shown in Fig. 5.3A). PL was not different between FRM and MM (65 to 67 mg.g DM^{-1}), and all other feeds contained approximately half that amount (27 to 37%) ($F_{6,7}$=221.6, P<0.001) (Table 5.2). Feed PCA1 scores correlated with a reduction in lipid efficiency ratio (R_{10}=-0.754, P=0.005) and whole body crude protein levels (R_{10}=-0.737, P=0.006) (Table 5.5) where the separation of two SQM data caused the strong correlation. The total neutral lipid content was highest in the SQM feed by percentage (83%) and lowest in the FrM (7%) (Fig. 5.2A). Neutral lipid:PL ratios were subsequently highest in SQM (5) and lowest in FrM (0.1) ($F_{6,7}$=171.5, P<0.001) (Table 5.1). The total neutral lipids content was highest in SQM (137 mg.g DM^{-1}) and FOL (93.4 mg.g DM^{-1}) and lowest in FrM (4.6 mg.g DM^{-1}) ($F_{6,7}$=1207.2, P<0.001) (Fig. 5.2B).

5.4.2 Digestive gland - percentage lipid class composition

The digestive gland lipid class composition was described on two axes (Fig. 5.1B), where PCA1 described 72% of the variation driven by different percentage compositions of PL, ST, WE, offset by DG and TAG (Fig. 5.1B). The percentage composition of WE was highest in TO (3%), lowest in FrM and absent from MM and CO ($F_{7,8}$=14.3, P=0.001) (Table 5.6). TAG content was highest in the FrM (37%) and lowest in TO, FO, SQM (1 to 4%). DG were absent in SQM and at the highest level in FOL and MM (4

and 3% respectively) ($F_{7,8}$=5.6, P=0.013) (Table 5.6). The second axis (PCA2, 19% of total variation) explained variation in digestive gland FFA levels (Fig 1B), that were, however, were not significantly different on a percentage basis ($F_{7,8}$=1.052, P=0.467) (Table 5.6). Digestive gland ST levels were significantly lower in FrM fed lobsters (2%) than TO the highest (4%) ($F_{7,8}$=4.5, P=0.026) (Table 5.6). The percentage of PL was highest in the SQM fed lobsters (62%) and lowest in FrM, MM, CO and FOL fed lobsters (29 to 41%) ($F_{7,8}$=7.8, P=0.005). The percentage of each lipid class in the digestive glands of initial lobsters was not significantly different to digestive glands of those fed formulated feeds (Table 5.6). The composition was most similar in composition to the digestive glands of lobsters fed FrM, although the initial digestive glands had a significantly higher WE content than the FrM fed lobsters (Fig. 5.4A). Decreasing DG and TAG, and increased PL, ST and WE in the digestive gland (Digestive gland PCA1 scores), were negatively correlated with protein productive values, lipid efficiency ratios and the total digestive gland and whole body lipid levels (Table 5.8).

5.4.3 Digestive gland - quantitative lipid class composition

When lipid classes were considered per g wet tissue, the digestive glands of SQM fed lobsters consistently contained the lowest levels of all classes. The variation in composition was explained by high WE content of FOL, TO (2 $mg.g^{-1}$) (PCA2, 18% total variation) (Fig 2B), that was significantly lowered in lobsters fed CO, FO, SQM, MM and FrM (0 to 0.6 $mg.g^{-1}$) ($F_{7,8}$=8.0, P=0.004) (Table 5.7). The majority of differences in lipid composition were explained by high levels of all other lipid classes, except WE (PCA1, 74%) (Fig 2B). The initial lobsters contained similar TAG to FrM, FOL and MM fed lobsters (46 $mg.g^{-1}$), and SQM, TO, FO, CO and the initial lobsters contained lower TAG (0.3 to 17 $mg.g^{-1}$) ($F_{7,8}$=8.7, P=0.003) (Table 5.7). The levels of FFA in digestive glands were similar between the initial lobsters and those fed FrM, MM, FOL and CO, while the SQM contained significantly less FFA ($F_{7,8}$=6.5, P=0.008). ST levels were significantly higher in the initial lobsters (3 $mg.g^{-1}$) than the SQM lobsters (0.9 $mg.g^{-1}$) ($F_{7,8}$=4.0, P=0.034). The digestive glands of initial lobsters contained similar PL to FrM, MM, CO and FOL content (27 to 40 $mg.g^{-1}$) and significantly more PL than FO, TO and SQM (14 to 18 $mg.g^{-1}$) ($F_{7,8}$=6.7, P=0.008)

(Table 5.7). Digestive gland PCA2 was positively correlated with the productive lipid value (R=0.935, P=0.005, n=12), the digestive gland lipid level (R10=0.962, P<0.001), whole body lipid level (R10=0.947, P<0.001) and the whole body crude protein level (R10=0.842, P=0.001) (Table 5.9).

5.4.4 Muscle tissue - percentage lipid class composition

The patterns in muscle tissue lipid composition of the formulated feed fed lobsters differed from the digestive gland (Fig 4A), where in muscle tissues TAG, FFA , ST and PL accounted for 54% of the total variation (PCA1, Fig. 5.3C). MM contained higher levels of PL (88%) than both SQM (81%) and the initial lobster muscle tissues (82%) (Table 5.8). FFA was highest in the initial lobsters (5%) with no difference between SQM and MM (2%) ($F_{2,3}$=25.3, P=0.013) (Table 5.8). PCA2 (39% variation) muscle tissue composition was characterised by high WE and low DG, however, the percentage compositions of WE, DG, TAG, and ST were not significantly different between the initial, and SQM and MM fed, lobsters (Table 5). There were no significant correlations between whole body tissue PCA scores and growth performance (Table 5.8).

5.4.5 Muscle tissue - quantitative lipid class composition

Muscle tissue PCA1 described 58% of the total variation in lipid class composition, and was driven by high loadings for TAG, FFA and DG (Fig 2C). The second factor, PCA2 explained 25% of the variation which was attributed to high ST and PL (Fig 2C). However the muscle content of WE, DG and ST was not significantly different by one-way ANOVA in muscle tissues (Table 5.9). TAG and FFA were lower in SQM (0.1 and 0.2 $mg.g^{-1}$ respectively) than MM and initial lobsters (0.2 and 0.5 $mg.g^{-1}$ respectively) (Table 5.9). PL was higher in the MM muscle tissues (14 $mg.g^{-1}$) than the initial lobsters (9 $mg.g^{-1}$) and SQM (5 $mg.g^{-1}$) (Table 5.9). The muscle tissue PCA1 was positively correlated with the same parameters as digestive gland PCA1; lipid efficiency ratio (R_{10}=0.903, P<0.001), digestive gland lipid (R=0.953, P<0.001, n=12), whole body crude lipid levels (R=0.930, P<0.001, n=12) and whole body crude protein (R=0.824, P<0.001, n=12) (Table 5.9).

5.4.6 Digestive gland and muscle tissue - neutral and polar lipids

The neutral lipid level as a percentage of total lipid in digestive glands was not different between SQM, FO and TO fed lobsters. However, the SQM (38%) was significantly lower than CO, FOL, MM, FrM and the initial lobsters (59 to 71%) ($F_{7,8}$=7.8, P=0.005) (Fig. 5.3A). Similarly, on a quantitative basis, the digestive glands of SQM, TO and FO fed lobsters contained less neutral lipid (9 to 17 $mg.g^{-1}$) than CO, FOL, MM fed and initial lobsters (49 to 67 $mg.g^{-1}$), while FrM contained the highest levels (87 $mg.g^{-1}$) ($F_{7,8}$=55.3, P<0.001). The digestive gland neutral/polar ratio did not differ between initial lobsters or those fed formulated feeds or FrM ($F_{7,8}$=3.3, 0.059) (Table 5.7).

Muscle tissues of MM fed lobsters had lower percentage of neutral lipid, and higher percentage of polar lipid content than SQM and initial lobsters (Table 5.8). However, the proportion of neutral lipids by weight was lower in SQM (1.2 $mg.g^{-1}$) than MM or initial muscle tissues (1.9 $mg.g^{-1}$) (Table 5.8, Fig. 5.2B). The ratio of neutral/polar lipids was lower in the MM muscle tissues (0.1) than the SQM or initial muscle tissues (0.2) (Table 5.9).

5.5 Discussion

The description of patterns in lipid composition using principal components analysis identified several key relationships between dietary lipid composition and weight gain, and the efficient use of ingested lipids to produce weight gain (LER). Digestive gland composition was correlated with the nutrient retention values for protein and lipid (PPV and PLV) and lipid levels in the lobster tissues. Biological explanations for these relationships are proposed.

5.5.1 Feed composition

The formulated feeds showed similar patterns in lipid class composition on both a percentage and quantitative ($mg.gDM^{-1}$) basis. TAG content was higher in SQM (101 $mg.g\ DM^{-1}$) than the other feeds. While squid mantle tissue is typically low in TAG (2% of total lipids), high levels of TAG in the digestive gland (75% of total lipids) have been

reported (Phillips et al., 2001). Phillips et al., (2001) also noted high levels of FFA (12%) in squid tissues and suggested elevated FFA levels are commonly associated with digestive gland enzymatic activity. High FFA may lower the efficiency of lipid absorption. High FFA levels are also used to indicate lipid oxidisation (Sasaki and Capuzzo, 1984; Guillaume and Métailler, 1999), and it remains possible that the quality of the squid meal may have been compromised during processing prior to feed formulation.

The PL composition of the FrM and MM feeds (93 and 43%, 67 and 65 mg.g DM^{-1}, respectively) was twice that of the formulated feeds. Murphy et al. (2000) reported 74% PL for *Mytilus edulis*. Fresh mussels used in the current study had recently spawned, causing a relative increase in the PL proportion of total lipids, however similar absolute quantities of PL were seen in FRM and MM. Squid meal contained high relative proportions of neutral lipids and low polar lipids, where the neutral/polar ratio (5) was nearly twice that of the other feeds. In contrast, fresh mussel was dominated by polar lipids and had a neutral/polar lipid ratio of 0.1.

5.5.2 Relationships between dietary and tissue lipid composition

The digestive gland and muscle tissues did not reflect the percentage lipid class composition of the feeds. However, there were general patterns that emerged between the tissues. ST levels were relatively similar in concentration and percentage level within tissues which is consistent with their structural role in tissues (Fraser, 1989). The digestive gland of FrM, FOL, CO and MM fed lobsters contained equivalent amounts of PL, FFA and TAG, while the initial lobsters had similar amounts of PL and FFA, but TAG was halved. The lipid content of FO, TO and SQM digestive glands were greatly reduced and they contained similar amounts of PL and FFA, with no TAG. The patterns in TAG composition correspond with observations of lipid storage droplets seen in histological sections of the same lobsters (Chapter 4), where very few lipid droplets were seen in FO, TO and SQM digestive glands. FFA levels in digestive glands were much higher than those present in feeds and most likely were associated with enzyme activity and lipid digestion within the digestive gland. PL in the digestive glands generally followed similar trends as the TAG content, but the initial and FrM fed lobsters were higher in TAG than the formulated feeds. American lobsters in good

nutritional condition accumulate TAG in the digestive gland and will catabolise TAG during starvation (Sasaki et al., 1986). It appears TAG plays the same role in juvenile *J. edwardsii.* The high PL and lower TAG content of the initial lobsters can be explained by their previous capture with depleted lipid reserves (Jeffs et al., 2002), and recent commencement of feeding with subsequent accumulation of TAG (Fraser, 1989). The lipid class composition of muscle tissue lipid class was similar between the initial lobsters and the SQM and MM fed lobsters, where PL and ST made up the greatest proportion in quantity and by percentage, as previously seen in *J. edwardsii* (Smith et al., 2004). Smaller amounts of FFA and DG were also present. The total amount of lipid, however, was greater in the MM fed lobster muscle tissue.

The neutral/polar lipid ratio by percentage were not related to the composition of feed, and remained constant among the muscle tissues whereas PL lipids dominated the total lipid, and varied greatly in the digestive gland. Muscle tissue neutral/PL ratios were 0.23 in SQM and initial lobsters, and 0.14 in MM, which were similar to reported ratios of 0.24 and 0.27 in *P. esculentus* (Chandumpai et al., 1991). The digestive gland of SQM fed lobsters contained the largest proportion of polar lipids, and smallest proportion of neutral lipids, indicating that neutral lipid reserves were absent, and structural polar lipids remained. Neutral/polar lipid ratios were shown to fall in response to starvation in *P. esculentus* (Chandumpai et al., 1991). FrM contained the largest proportion of neutral lipid by percentage and weight, indicating abundant storage of lipids in addition to the structural polar lipid fraction which agrees with wild adult *J. lalandii* (Cockcroft, 1997). The average digestive gland neutral/polar ratio ranged between 2.50 (FrM) and 0.61 (SQM) in the present study, which is similar to ratios in *P. esculentus* which were highest at 2.53 and lowest at 0.24 (after 21 days starvation) (Chandumpai et al., 1991). D'Abramo et al., (1980) reported that the inclusion of tuna oil in feeds caused a decrease in the neutral lipid component of the digestive gland in *Homarus americanus*, and suggested that PUFA were less likely to be stored as depot fat (neutral lipid), and subsequently cause a decrease in the digestive gland neutral lipid content (D'Abramo, 1997). This preferential exclusion of PUFA from neutral lipid stores may explain the low neutral/polar digestive gland lipid levels in FO (0.99) and TO (1.13).

5.5.3 Relationships between lipid class composition and growth

High productive protein values (PPV), productive lipid values (PLV) and high tissue lipid content, were strongly correlated with high percentage levels of DG and TAG and low levels of PL, ST and WE in the digestive gland. This confirms that TAG storage in juvenile *J. edwardsii* is related to high growth rates, and in particular the effective incorporation of ingested protein (PPV) and lipid (PLV) into tissues. The strong correlation of DG with PPV and PLV in juvenile *J. edwardsii* suggests DG in the digestive gland is associated with high growth and lipid performance of *J. edwardsii*. DG may be implicated as a direct energy substrate or as an intermediate in biosynthetic pathways e.g. TAG synthesis or catabolism (D'Abramo et al., 1980; Chandumpai et al., 1991), and correlation with growth rates may be a function of storage or higher biosynthetic rates or digestion in fast growing lobsters. Chandumpai et al., (1991) noted that TAG and DG both decreased in *P. esculentus* under starvation, suggesting their importance as energy substrates, whereas the proportion of ST increased.

The main relationship between quantitative lipid class composition and biological parameters were evident in the digestive gland. The strong correlation between high protein productive values, lipid efficiency ratios and the total digestive gland and whole body lipid and digestive gland content were attributed to the gross deposition of all lipid classes except WE. There appears no relationship between WE and deposition of lipid in body tissues and growth efficiency (as PPV and LER). WE is not often seen in high quantities in tissues of *J. edwardsii* phyllosoma (Smith et al., 2003) or puerulus (Jeffs et al., 2001) and has been reported absent in adult *J. edwardsii* (Nichols et al., 1988). Digestive gland PCA1 may provide a more generalised description of lipid deposition, equivalent to lipid condition indices reported in *J. lalandii* (Cockcroft, 1997), where increased digestive gland lipid storage was related to growth, corresponding with the increased lipid efficiency ratio and productive protein values in the present study.

Dietary provision of PL and ST with low TAG levels in feeds were positively correlated with high productive protein values, but low lipid efficiency ratio. This was demonstrated mainly by the good growth performance of the FrM and MM feeds, although this trend was not found among the formulated feeds, which were high in TAG. Penaeid prawns fed dietary PL increase both energy deposition and the amount of

energy available for growth by improving the availability of EFA in the TAG components of feeds (Kontara, 1997). The persistent superiority in growth rates of the FrM fed lobsters over formulated feed fed lobsters suggests the provision of PL in feeds supports better growth and TAG deposition, than providing dietary TAG. The replacement of FO with soybean lecithin in FOL, increased the digestive gland lipid deposition and non-significantly increased growth rates. Replacing TAG with PL into lobster feeds, and assessing the effective provision of EFA deserves further research.

5.6 Conclusion

The lipid class profiles of lobster tissues were affected by dietary treatment, but do not necessarily reflect the lipid class composition of diets. TAG and potentially DG are the main lipid storage forms in juvenile *J. edwardsii* and their accumulation in the digestive gland are strongly correlated with productive protein value and lipid content of body tissues. This finding is confirmed by both individual lipid class comparison and the histology results for lipid deposition (Chapter 4). Fraser (1989) suggested the application of TAG/ST ratios as an indicator of nutritional condition in wild *H. americanus*. The results of the present study support the application of this index in *J. edwardsii*, where ST remained constant in tissues and TAG varied greatly with dietary treatment. Jeffs et al. (2002), however, suggested that PL may a dominant role in energy storage in both larval and adult *J. edwardsii.* The reduction of PL may form a secondary substrate of energy utilisation after neutral lipid reserves are depleted, which may explain the decrease in PL observed in the slow growing SQM fed lobsters, where TAG was absent. FrM and MM appeared to present beneficial lipid profiles for feeds where high PL and ST, and low TAG were correlated with the highest productive protein value. Future research on juvenile lobsters should aim to improve the PL profile of these feeds, and to use ingredients that will promote the deposition of TAG to improve current lobster growth rates.

Table 5.1. Percentage lipid class composition as a percentage of total lipid (mean and standard deviation, df=6,7) of formulated feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and fresh mussel (FrM) in a 10 week growth trial.

Lipid class		CO	FOL	FO	TO	SQM	MM	FRM	F	P
Wax ester[1]	mean	0.5 ^{a}	0.2 ^{a}	0.3 ^{a}	0.3 ^{a}	1.9 ^{b}	0.0 ^{a}	0.2 ^{a}	56.0	**<0.001**
	S.D.	0.1	0.0	0.1	0.0	0.3	0.0	0.1		
Triglyceride	mean	69.2 ^{d}	66.3 ^{d}	68.2 ^{d}	67.2 ^{d}	61.3 ^{c}	51.9 ^{b}	0.3 ^{a}	710.3	**<0.001**
	S.D.	0.0	1.4	2.7	0.2	1.4	1.0	0.1		
Free fatty acid	mean	2.5 ^{a}	3.6 ^{a}	3.3 ^{a}	2.6 ^{a}	16.9 ^{b}	2.0 ^{a}	3.6 ^{a}	249.7	**<0.001**
	S.D.	0.1	0.2	0.6	0.2	0.8	0.4	0.5		
Diglyceride	mean	0.2 ^{ab}	0.5 ^{b}	0.3 ^{ab}	0.4 ^{ab}	2.2 ^{c}	0.2 ^{ab}	0.0 ^{a}	85.1	**<0.001**
	S.D.	0.0	0.2	0.1	0.1	0.2	0.1	0.0		
Sterol	mean	1.4 ^{a}	1.3 ^{a}	1.7 ^{a}	1.9 ^{ab}	1.1 ^{a}	2.5 ^{b}	2.6 ^{b}	12.5	**0.002**
	S.D.	0.2	0.1	0.3	0.3	0.2	0.1	0.3		
Phospholipid (PL)	mean	26.2 ^{b}	28.1 ^{b}	26.2 ^{b}	27.7 ^{b}	16.6 ^{a}	43.4 ^{c}	93.4 ^{d}	880.3	**<0.001**
	S.D.	0.0	1.9	1.9	0.7	0.6	1.3	0.9		
Neutral lipid[2]	mean	73.8 ^{c}	71.9 ^{c}	73.8 ^{c}	72.3 ^{c}	83.4 ^{d}	56.6 ^{b}	6.6 ^{a}	880.3	**<0.001**
	S.D.	0.0	1.9	1.9	0.7	0.6	1.3	0.9		
Neutral:PL ratio[3]	mean	2.8 ^{c}	2.6 ^{c}	2.8 ^{c}	2.6 ^{c}	5.0 ^{d}	1.3 ^{b}	0.1 ^{a}	171.5	**<0.001**
	S.D.	0.0	0.2	0.3	0.1	0.2	0.1	0.0		

Means that are not significantly different share similar superscripts. Bold case indicates significance by oneway ANOVA (F=F ratio, P=significance which was accepted at α=0.05).

[1] includes hydrocarbons.

[2] Neutral lipids include wax esters, triglycerides, free fatty acids, diglycerides and sterols.

[3] Ratio of neutral lipids to phospholipid (polar lipid).

Table 5.2. Quantitative lipid class composition (mg lipid.g DM^{-1}) (mean and standard deviation, df=6,7) of formulated feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and fresh mussel (FrM) in a 10 week growth trial.

Lipid class		CO	FOL	FO	TO	SQM	MM	FRM	F	P
Wax ester[1]	mean	0.6 ^{a}	0.3 ^{a}	0.4 ^{a}	0.3 ^{a}	3.1 ^{b}	0.1 ^{a}	0.1 ^{a}	65.4	**<0.001**
	S.D.	0.1	0.0	0.1	0.0	0.5	0.1	0.0		
Triglyceride	mean	81.2 ^{b}	86.1 ^{b}	83.9 ^{b}	82.3 ^{b}	100.7 ^{c}	80.6 ^{b}	0.2 ^{a}	698.7	**<0.001**
	S.D.	0.0	1.8	3.3	0.3	2.3	1.5	0.1		
Free fatty acid	mean	2.9 ^{a}	4.7 ^{a}	4.0 ^{a}	3.1 ^{a}	27.7 ^{b}	3.1 ^{a}	2.5 ^{a}	381.1	**<0.001**
	S.D.	0.1	0.3	0.8	0.3	1.3	0.6	0.3		
Diglyceride	mean	0.3 ^{a}	0.6 ^{a}	0.3 ^{a}	0.5 ^{a}	3.6 ^{b}	0.3 ^{a}	0.0 ^{a}	122.3	**<0.001**
	S.D.	0.1	0.2	0.1	0.1	0.3	0.1	0.0		
Sterol	mean	1.6 ^{a}	1.7 ^{a}	2.0 ^{a}	2.3 ^{a}	1.8 ^{a}	3.9 ^{b}	1.8 ^{a}	16.9	**0.001**
	S.D.	0.2	0.2	0.4	0 4	0.3	0.1	0.2		
Phospholipid (PL)	mean	30.8 ^{ab}	36.6 ^{b}	32.3 ^{ab}	33.9 ^{b}	27.2 ^{a}	67.3 ^{c}	65.0 ^{c}	221.6	**<0.001**
	S.D.	0.0	2.5	2.3	0.8	0.9	2.1	0.6		
Neutral lipids[2]	mean	86.6 ^{b}	93.4 ^{c}	90.7 ^{ab}	88.6 ^{ab}	137.0 ^{d}	87.9 ^{ab}	4.6 ^{a}	1207.2	**<0.001**
	S.D.	0.0	2.5	2.3	0.8	0.9	2.1	0.6		
Neutral:PL ratio[3]	mean	2.8 ^{c}	2.6 ^{c}	2.8 ^{c}	2.6 ^{c}	5.0 ^{d}	1.3 ^{b}	0.1 ^{a}	171.5	**<0.001**
	S.D.	0.0	0.2	0.3	0.1	0.2	0.1	0.0		

Means that are not significantly different share similar superscripts. Bold case indicates significance by oneway ANOVA (F=F ratio, P=significance which was accepted at α=0.05).

[1] includes hydrocarbons.

[2] Neutral lipids include wax esters, triglycerides, free fatty acids, diglycerides and sterols.

[3] Ratio of neutral lipids to phospholipid (polar lipid).

Table 5.3. Percentage lipid class composition (mean and standard deviation, df=7,8) of juvenile lobster *Jasus edwardsii* digestive glands after feeding on formulated feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM), fresh mussel (FrM) in a 10 week growth trial and digestive glands from lobsters from the initial population (Initial).

Lipid class		CO	FOL	FO	TO	SQM	MM	FRM	Initial	F	P
Wax ester[1]	mean	0.0 ^{a}	1.0 ^{ab}	1.8 ^{bc}	3.2 ^{c}	1.2 ^{ab}	0.0 ^{a}	0.2 ^{a}	2.0 ^{bc}	14.3	**0.001**
	S.D.	0.0	1.0	0.0	0.6	0.2	0.0	0.3	0.0		
Triglyceride	mean	20.7 ^{ab}	31.2 ^{ab}	2.8 ^{a}	3.7 ^{a}	1.2 ^{a}	27.7 ^{ab}	37.1 ^{b}	13.2 ^{ab}	5.6	**0.013**
	S.D.	12.7	14.0	0.3	1.1	1.7	5.2	5.9	11.8		
Free fatty acid	mean	33.2	31.6	40.5	40.8	31.6	34.7	28.5	41.0	1.052	.467
	S.D.	13.1	4.7	3.2	3.2	1.4	1.7	11.0	5.2		
Diglyceride	mean	2.1 ^{ab}	3.6 ^{b}	0.8 ^{ab}	1.2 ^{ab}	0.0 ^{a}	3.3 ^{b}	2.9 ^{ab}	1.2 ^{ab}	5.6	**0.013**
	S.D.	0.4	1.2	0.4	0.6	0.0	1.0	1.3	0.3		
Sterol	mean	2.7	2.3	3.8	4.1	3.8	2.4	2.0	2.9	4.5	**0.026**
	S.D.	0.3	0.8	0.4	0.1	0.7	0.4	0.8	0.2		
Phospholipid (PL)	mean	41.4 ^{a}	30.3 ^{a}	50.4 ^{ab}	47.1 ^{ab}	62.2 ^{b}	31.9 ^{a}	29.3 ^{a}	39.8 ^{a}	7.8	**0.005**
	S.D.	1.2	8.8	4.3	4.2	3.6	7.5	6.8	6.0		
Neutral lipids[2]	mean	58.6 ^{b}	69.7 ^{b}	49.6 ^{ab}	52.9 ^{ab}	37.8 ^{a}	68.1 ^{b}	70.7 ^{b}	60.2 ^{b}	7.8	**0.005**
	S.D.	1.2	8.8	4.3	4.2	3.6	7.5	6.8	6.0		
Neutral:PL ratio[3]	mean	1.4	2.4	1.0	1.1	0.6	2.2	2.5	1.5	3. 3	0.059
	S.D.	0.1	1.0	0.2	0.2	0.1	0.8	0.8	0.4		

Means that are not significantly different share similar superscripts. Bold case indicates significance by oneway ANOVA (F=F ratio, P=significance which was accepted at α=0.05).

[1] includes hydrocarbons.

[2] Neutral lipids include wax esters, triglycerides, free fatty acids, diglycerides and sterols.

[3] Ratio of neutral lipids to phospholipid (polar lipid).

Table 5.4. Quantitative lipid class composition (mg lipid.g wet tissue^{-1}) (mean and standard deviation, df=7,8) of juvenile lobster *Jasus edwardsii* digestive glands after feeding on formulated feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM), fresh mussel (FrM) in a 10 week growth trial and digestive glands from lobsters from the initial population (Initial).

Lipid class		CO	FOL	FO	TO	SQM	MM	FRM	Initial	F	P
Wax ester[1]	mean	0.0 ^{a}	0.9 ^{ab}	0.6 ^{a}	1.2 ^{ab}	0.3 ^{a}	0.0 ^{a}	0.2 ^{a}	2.0 ^{b}	8.0	**0.004**
	S.D.	0.0	0.9	0.0	0.2	0.1	0.0	0.3	0.0		
Triglyceride	mean	17.1 ^{a}	28.2 ^{ab}	1.0 ^{a}	1.3 ^{a}	0.3 ^{a}	27.4 ^{ab}	45.8 ^{b}	13.2 ^{a}	8.7	**0.003**
	S.D.	10.5	12.6	0.1	0.4	0.4	5.1	7.3	11.8		
Free fatty acid	mean	27.6 ^{abc}	28.6 ^{abc}	14.2 ^{ab}	14.9 ^{ab}	7.1 ^{a}	34.3 ^{bc}	35.1 ^{bc}	41.0 ^{c}	6.5	**0.008**
	S.D.	10.9	4.2	1.1	1.2	0.3	1.7	13.5	5.2		
Diglyceride	mean	1.7 ^{abc}	3.2 ^{bc}	0.3 ^{a}	0.4 ^{ab}	0.0 ^{a}	3.3 ^{bc}	3.6 ^{c}	1.2 ^{abc}	7.2	**0.006**
	S.D.	0.3	1.1	0.1	0.2	0.0	1.0	1.6	0.3		
Sterol	mean	2.2 ^{ab}	2.1 ^{ab}	1.3 ^{ab}	1.5 ^{ab}	0.9 ^{a}	2.4 ^{ab}	2.4 ^{ab}	2.9 ^{b}	4.0	**0.034**
	S.D.	0.3	0.7	0.1	0.0	0.2	0.4	1.0	0.2		
Phospholipid (PL)	mean	34.4 ^{abc}	27.3 ^{abc}	17.6 ^{ab}	17.2 ^{ab}	13.9 ^{a}	31.5 ^{abc}	36.2 ^{bc}	39.8 ^{c}	6.7	**0.008**
	S.D.	1.0	7.9	1.5	1.5	0.8	7.4	8.4	6.0		
Neutral lipids[2]	mean	48.6 ^{b}	63.0 ^{b}	17.4 ^{a}	19.4 ^{a}	8.5 ^{a}	67.4 ^{b}	87.1 ^{c}	60.2 ^{b}	55.3	**<0.001**
	S.D.	1.0	7.9	1.5	1.5	0.8	7.4	8.4	6.0		
Neutral:PL ratio[3]	mean	1.4	2.4	1.0	1.1	0.6	2.2	2.5	1.5	3.3	0.059
	S.D.	0.1	1.0	0.2	0.2	0.1	0.8	0.8	0.4		

Means that are not significantly different share similar superscripts. Bold case indicates significance by oneway ANOVA (F=F ratio, P=significance which was accepted at α=0.05).

[1] includes hydrocarbons.

[2] Neutral lipids include wax esters, triglycerides, free fatty acids, diglycerides and sterols.

[3] Ratio of neutral lipids to phospholipid (polar lipid).

Table 5.5. Percentage lipid class composition (mean and standard deviation, df=2,3) of juvenile lobster *Jasus edwardsii* muscle tissue after feeding on formulated feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM), fresh mussel (FrM) in a 10 week growth trial and muscle tissue from lobsters from the initial population (Initial).

Lipid class		SQM	MM	Initial	F	P
Wax ester[1]	mean	0.3	0.1	0.4	1.5	.357
	S.D.	0.4	0.1	0.1		
Triglyceride	mean	1.8	1.6	2.4	8.4	0.59
	S.D.	0.3	0.0	0.0		
Free fatty acid	mean	3.1[a]	2.4[a]	4.9[b]	25.3	**0.013**
	S.D.	0.3	0.3	0.5		
Diglyceride	mean	0.8	0.4	0.6	0.286	0.769
	S.D.	0.9	0.1	0.1		
Sterol	mean	12.8	7.6	10.2	7.221	0.071
	S.D.	2.0	0.6	1.0		
Phospholipid (PL)	mean	81.3[a]	87.9[b]	81.5[a]	14.0	**0.030**
	S.D.	2.0	0.3	1.5		
Neutral lipids[3]	mean	18.7[b]	12.1[a]	18.5[b]	14.0	**0.030**
	S.D.	2.0	0.3	1.5		
Neutral:PL ratio	mean	0.2[b]	0.1[a]	0.2[b]	12.1	**0.037**
	S.D.	0.0	0.0	0.0		

Means that are not significantly different share similar superscripts. Bold case indicates significance by oneway ANOVA (F=F ratio, P=significance which was accepted at α=0.05).

[1] includes hydrocarbons.

[2] Neutral lipids include wax esters, triglycerides, free fatty acids, diglycerides and sterols.

[3] Ratio of neutral lipids to phospholipid (polar lipid).

Table 5.6. Quantitative lipid class composition (mg lipid.g wet tissue^{-1}) (mean and standard deviation, df=2,3) of juvenile lobster *Jasus edwardsii* muscle tissue after feeding on formulated feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM), fresh mussel (FrM) in a 10 week growth trial and muscle tissue from lobsters from the initial population (Initial).

Lipid class		SQM	MM	Initial	F	P
Wax ester[1]	mean	0.0	0.0	0.0	2.7	0.211
	S.D.	0.0	0.0	0.0		
Triglyceride	mean	0.1 ^{a}	0.2 ^{b}	0.2 ^{b}	68.5	**0.003**
	S.D.	0.0	0.0	0.0		
Free fatty acid	mean	0.2 ^{a}	0.4 ^{b}	0.5 ^{b}	31.5	**0.010**
	S.D.	0.0	0.0	0.1		
Diglyceride	mean	0.0	0.1	0.1	0.057	0.946
	S.D.	0.1	0.0	0.0		
Sterol	mean	0.8	1.2	1.1	5.937	0.091
	S.D.	0.1	0.1	0.1		
Phospholipid (PL)	mean	5.2 ^{a}	13.6 ^{c}	8.6 ^{b}	2640.0	**<0.001**
	S.D.	0.1	0.0	0.2		
Neutral lipids[2]	mean	1.2 ^{a}	1.9 ^{b}	1.9 ^{b}	24.9	**0.014**
	S.D.	0.1	0.0	0.2		
Neutral:PL ratio[3]	mean	0.2 ^{b}	0.1 ^{a}	0.2 ^{b}	12.1	**0.037**
	S.D.	0.0	0.0	0.0		

Means that are not significantly different share similar superscripts. Bold case indicates significance by oneway ANOVA (F=F ratio, P=significance which was accepted at α=0.05).

[1] includes hydrocarbons.

[2] Neutral lipids include wax esters, triglycerides, free fatty acids, diglycerides and sterols.

[3] Ratio of neutral lipids to phospholipid (polar lipid).

Table 5.7. Growth performance and nutrient retention means and standard errors (S.E.) of juvenile *J. edwardsii* that were significantly affected by feed by oneway ANOVA (df=5,1) from Chapter 3.

	CO	FOL	FO	TO	SQM	MM	F	P
Growth performance								
PPV[1]	6.47^{ab}	10.89^{b}	8.63^{ab}	9.90^{b}	3.12^{a}	12.69^{b}	5.864	**0.011**
S.E.	*1.52*	*0.24*	*1.50*	*1.30*	*0.87*	*1.35*		
PLV[2]	2.68^{ab}	3.42^{b}	1.66^{ab}	1.63^{ab}	0.13^{a}	3.80^{b}	11.481	**0.001**
S.E.	*1.01*	*0.81*	*0.60*	*0.26*	*0.21*	*0.35*		
LER[3]	184.83^{ab}	245.21^{b}	220.22^{ab}	272.42^{b}	104.03^{a}	209.87^{ab}	4.001	**0.034**
S.E.	*24.73*	*27.08*	*30.51*	*30.45*	*27.35*	*17.48*		
Digestive gland								
Lipid	8.30^{bc}	9.03^{bc}	3.50^{ab}	3.66^{ab}	2.24^{a}	9.89^{c}	8.888	**0.003**
S.E.	*2.16*	*2.27*	*0.28*	*0.39*	*0.20*	*1.13*		
Whole body								
Lipid	1.26^{bc}	1.28^{bc}	0.87^{ab}	0.76^{ab}	0.64^{a}	$1\ 55^{c}$	8.242	**0.004**
S.E.	*0.21*	*0.30*	*0.10*	*0.02*	*0.01*	*0 11*		
Crude protein	12.08^{ab}	13.50^{b}	12.53^{b}	11.98^{ab}	10.06^{a}	13.48^{b}	7.040	**0.006**
S.E.	*0.65*	*0 71*	*0.40*	*0.29*	*0 41*	*0.38*		

Means that are not significantly different share similar superscripts. Bold case indicates significance by oneway ANOVA (F=F ratio, P=significance which was accepted at α=0.05).

[1]Productive protein value (PPV) = initial protein-final protein/protein consumed*100

[2]Productive lipid value (PLV) = initial protein-final protein/protein consumed*100

[3]Lipid efficiency ratio (LER) = weight gain/lipid consumed*100

Values that are not significantly different share a common superscript.

Table 5.8. Pearson correlation coefficient matrix for PCA factor scores of 1 (PCA1) and 2 (PCA2) for digestive gland (DG), muscle tissues (MT) and feed (F) percentage lipid classes against selected growth parameters for lobsters fed all feeds with significant correlations indicated in bold (n=12, df=10).

	Feed PCA1		Feed PCA2		Digestive gland PCA1		Digestive gland PCA2		Whole body PCA1		Whole body PCA2	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Weight gain	**0.850**	**<0.001**	-0.388	0.171	-0.569	0.034	-0.086	0.771	-0.715	0.285	-0.573	0.427
PPV	0.422	0.172	**-0.755**	**0.005**	**-0.761**	**0.004**	0.457	0.136	-0.628	0.372	-0.673	0.327
PLV	0.237	0.458	-0.669	0.017	**-0.965**	**<0.001**	0.091	0.778	-0.621	0.379	-0.670	0.330
LER	**-0.739**	**0.003**	-0.463	0.095	-0.043	0.885	0.658	0.011	-0.916	0.010	-0.374	0.465
DG lipid	0.587	0.027	-0.428	0.127	**-0.822**	**<0.001**	-0.398	0.159	0.184	0.727	-0.516	0.295
MT lipid	0.551	0.041	-0.446	0.110	**-0.820**	**<0.001**	-0.383	0.176	-0.351	0.495	-0.696	0.125
MT crude protein	0.063	0.831	**-0.743**	**0.002**	-0.679	0.008	0.030	0.919	-0.174	0.741	-0.773	0.072

Significance accepted at $\alpha = 0.006$ after Bonferroni correction, bold case indicates a significant correlation
PPV=productive protein value = g protein gained/g protein consumed*100
PLV=productive lipid value = g lipid gained/g lipid consumed*100
LER=lipid efficiency ratio = g weight gain/g lipid consumed*100
MT = muscle tissue

Table 5.9. Pearson correlation coefficient matrix for PCA factor scores 1 (PCA1) and 2 (PCA2) for feed (F), digestive gland (DG), Whole body tissue without digestive gland (WB) expressed as mg lipid.g wet tissue^{-1} against selected growth parameters for lobsters fed formulated feeds only with significant correlations indicated in bold (n=12, df=10).

	Feed PCA1		Feed PCA2		Feed PCA3		Digestive gland PCA1		Digestive gland PCA2		Whole body PCA1		Whole body PCA2	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Weight gain	-0.673	0.016	-0.062	0.848	0.396	0.202	0.507	0.093	0.360	0.250	0.439	0.153	0.016	0.960
PPV	-0.654	0.021	-0.224	0.485	0.588	0.044	0.664	0.019	0.134	0.679	0.601	0.039	0.229	0.474
PLV	-0.607	0.036	-0.314	0.320	0.402	0.196	**0.935**	**<0.001**	-0.320	0.311	**0.903**	**<0.001**	0.316	0.317
LER	**-0.754**	**0.005**	0.306	0.333	0.115	0.721	0.331	0.294	0.538	0.071	0.271	0.394	-0.293	0.356
DG lipid	-0.477	0.117	-0.373	0.232	0.302	0.340	**0.962**	**<0.001**	-0.462	0.131	**0.953**	**<0.001**	0.336	0.286
WB lipid	-0.445	0.147	-0.457	0.135	0.422	0.172	**0.947**	**<0.001**	-0.563	0.057	**0.930**	**<0.001**	0.466	0.126
WB crude protein	**-0.737**	**0.006**	-0.161	0.618	0.404	0.193	**0.842**	**0.001**	-0.044	0.892	**0.824**	**0.001**	0.141	0.661

Significance accepted at α=0.006 after Bonferroni correction, bold case indicates a significant correlation
PPV=productive protein value=g protein gained/g protein consumed*100
PLV=productive lipid value = g lipid gained/g lipid consumed*100
LER=lipid efficiency ratio = g weight gain/g lipid consumed*100
MT = muscle tissue

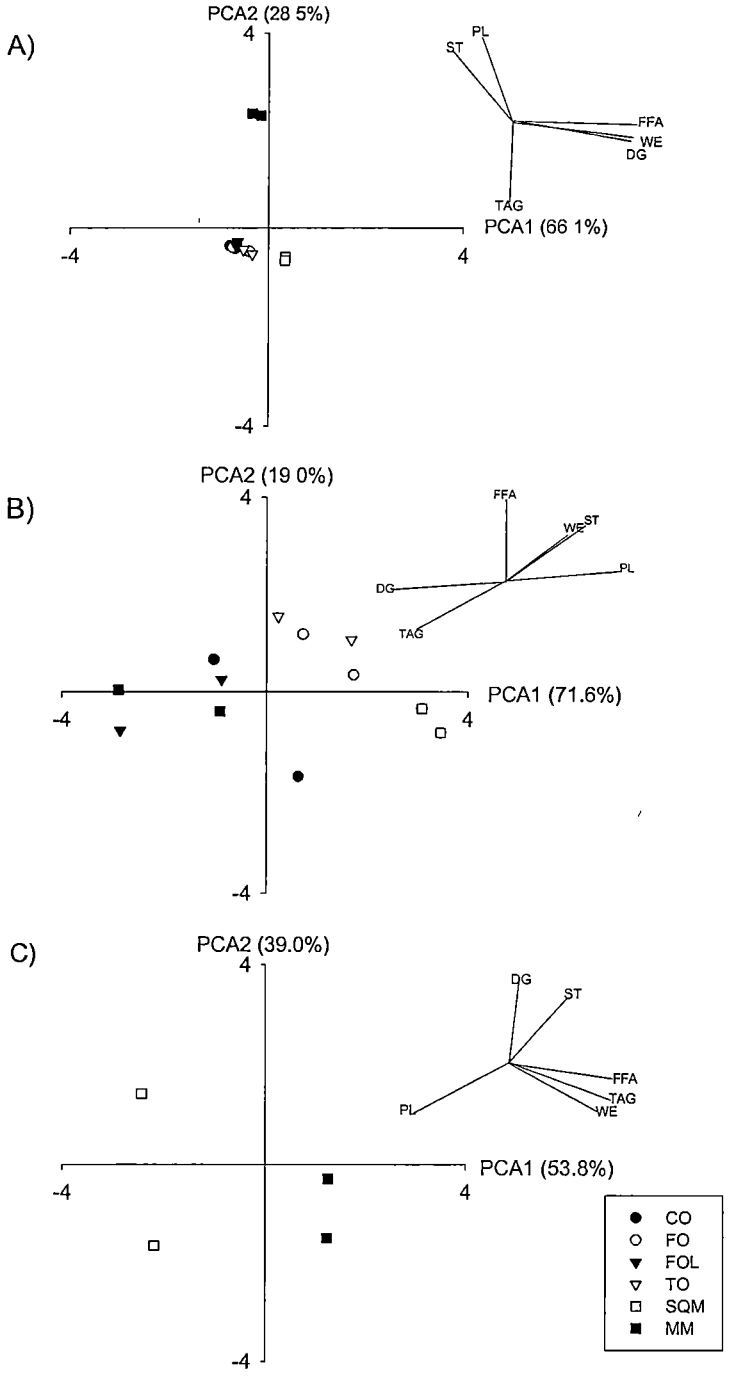

Figure 5.1. Individual lobster PCA scores explaining patterns in percentage lipid composition of **A)** feed PCA1 (66% total variation) and PCA2 (29% total variation), **B)** digestive gland lipid PCA1 (72% total variation) and PCA2 (19% total variation) and **C)** muscle tissue PC factors PCA1 (54% total variation) and PCA2 (40% total variation) after feeding formulated feeds containing canola oil (CO), fish oil (FO), fish oil and lecithin (FOL), tuna oil (TO), squid meal (SQM) and mussel meal (MM) for 10 weeks.

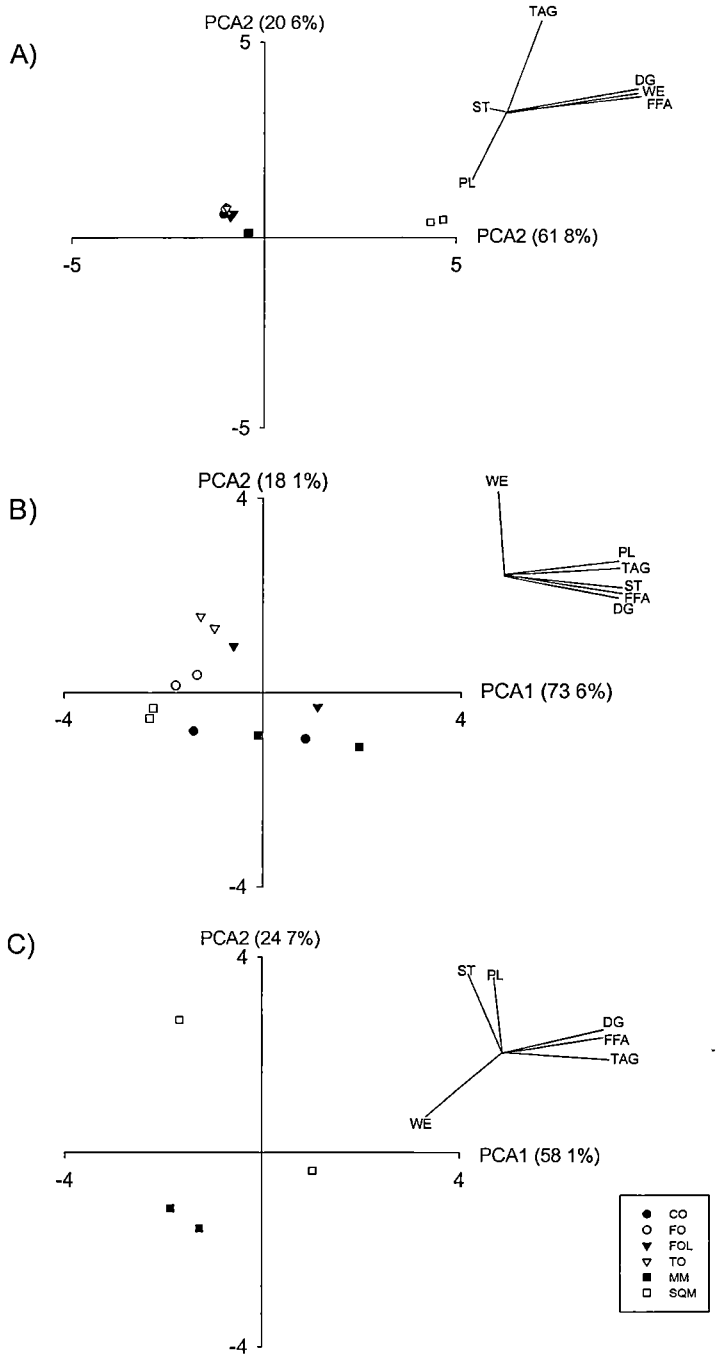

Figure 5.3. Individual lobster PCA scores for explaining patterns in percentage lipid composition of **A)** feed lipid class composition PCA1 (61% total variation) and PCA2 (21% total variation), **B**) digestive gland lipid class PCA1 (74 % total variation) and PCA2 (18% total variation) and **C**) muscle tissue lipid class PCA1 (58% total variation) and PCA2 (25% total variation) after feeding formulated feeds containing canola oil (CO), fish oil (FO), fish oil and lecithin (FOL), tuna oil (TO), squid meal (SQM) and mussel meal (MM) for 10 weeks.

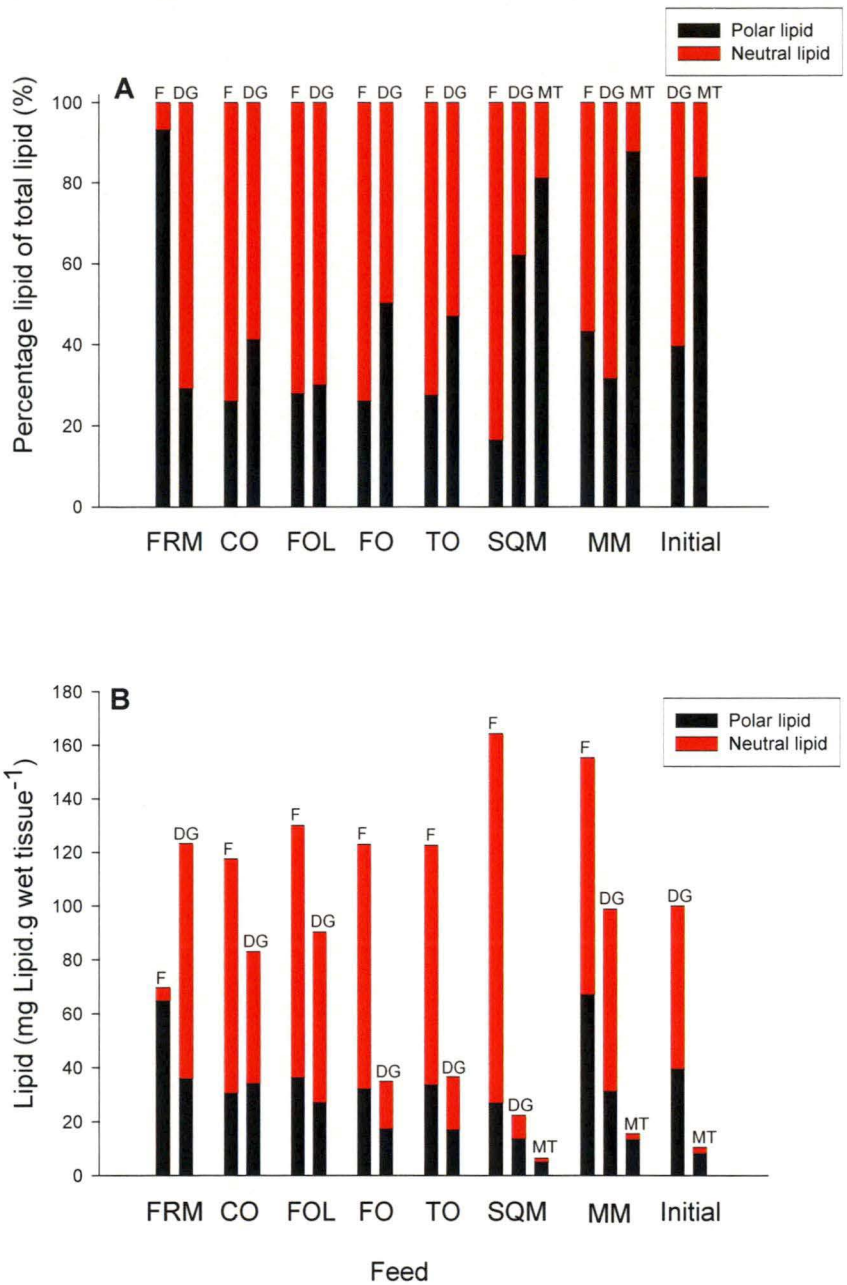

Figure 5.2 Polar and neutral lipid classes of feed (F), digestive gland (DG) and muscle tissue (MT) as a percentage of total lipids (A) and mg lipid class per g wet weight of digestive gland and muscle tissue or g feed (DM)(B) in juvenile *J. edwardsii* after feeding for 10 weeks. Fresh mussel fed lobsters and initial compositions are included for reference. Lobsters (n=5) from within each formulated feed treatment (n=3) and fresh mussel feed (n=2) were pooled and analysed in duplicate for all samples.

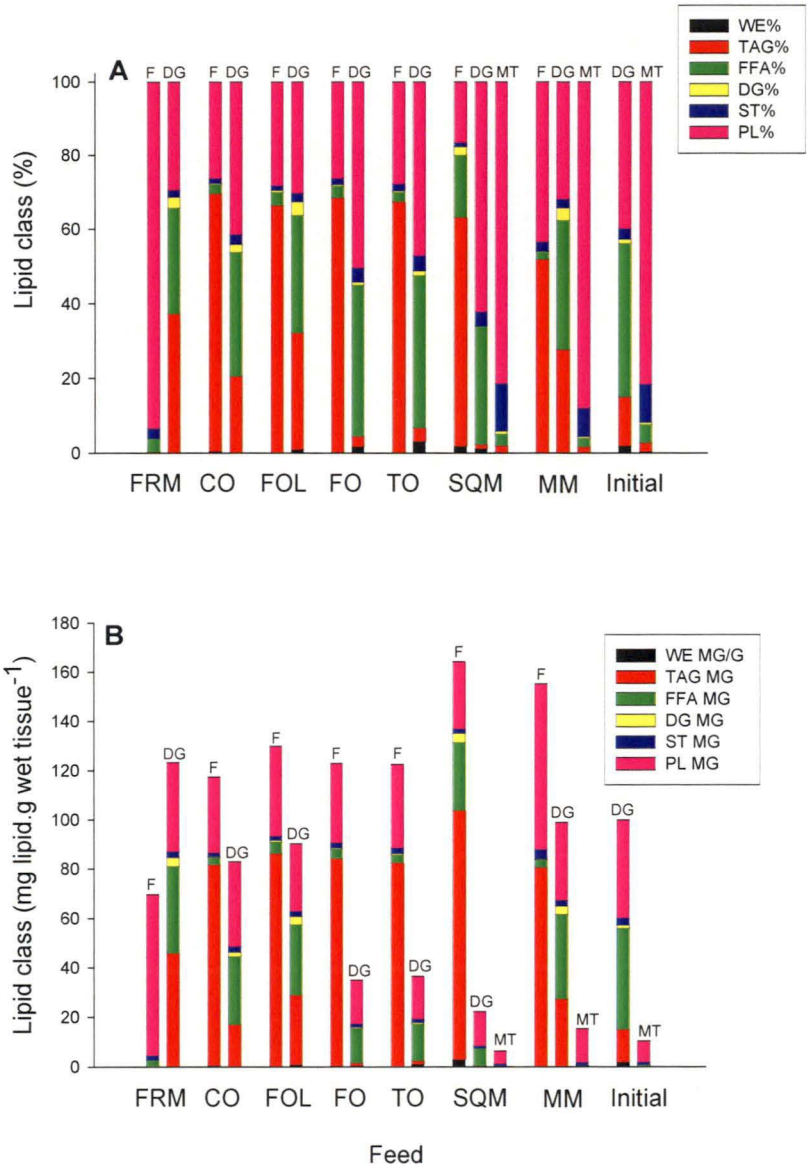

Figure 5.4. Lipid class composition of feed (F), digestive gland (DG) and muscle tissue (MT) as a percentage of total lipids (A) and mg lipid class per g wet weight of digestive gland and muscle tissue or g feed (DM)(B) in juvenile *J. edwardsii* after feeding for 10 weeks. Fresh mussel fed lobsters and initial compositions are included for reference. Lobsters (n=5) from within each formulated feed treatment (n=3) and fresh mussel feed (n=2) were pooled and analysed in duplicate for all samples.

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Chapter 6

Digestive gland and muscle fatty acid composition of juvenile lobster in response to different dietary oil sources

6.1 Abstract

Experimental lobster feeds are currently based on fish meal and fish oil formulations, and although good survival and growth up to that of lobsters fed fresh blue mussels has been achieved, varying dietary composition of feeds has not increased growth to that of natural food. This experiment assessed the performance of six formulated feeds containing commercial ingredients supplying the endogenous and exogenous lipids: fish oil, fish oil with added lecithin, canola oil, tuna oil, mussel meal, squid meal. Three groups of 15 post-larval lobsters were allocated one of the six test feeds. Two tanks were fed a reference feed of freshly opened blue mussels. Lobsters were fed daily to excess, and weight measured every three weeks. At the end of the experiment final weights of whole body and digestive gland were recorded and both tissues removed and immediately frozen for fatty acid analyses. There were no significant differences in survival, weight gain or specific growth rate among the lobsters. The main fatty acids in both digestive gland tissues and muscle tissues were eicosapentaenoic acid (20:5n-3), docosahexaenoic acid (22:6n-3), palmitic acid (16:0) and the oleic acid (18:1n-9). Although growth was not affected by diet there were significant differences in lipid composition. Levels of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids were significantly lower in the digestive glands of squid meal fed lobsters, and highest in lobsters fed fresh mussel. Digestive gland fatty acid compositions reflected the composition of the feed, whereas the muscle tissue fatty acid composition was independent of diet. The addition of dietary phospholipids (soybean lecithin) to fish meal fish oil based feeds did not increase growth rates, but dramatically changed the fatty acid profile and digestive gland lipid levels. There is considerable potential to replace fish oils with alternative marine meals with limited effects on muscle tissue compositions and growth rates.

6.2 Introduction

Monogastric animals, including marine animals, typically adopt characteristics of the fatty acid composition of their feed, as ingested lipids are stored in fat depots and incorporated into tissues (Jakobsen, 1999). The incorporation of dietary fatty acids has been recently used to determine food chain interactions by identifying signature fatty acids in squid (Phillips et al., 2001), penaeid prawns (Bottino et al., 1980) and *J. edwardsii* phyllosoma (Jeffs et al., 2004). In cultured crustacean species, studies into the effects of dietary lipids on tissue composition have focused largely on tropical penaeid species (Middleditch et al., 1980) including *Penaeus monodon*, (Merican and Shim, 1994; Deering and Hewitt, 1995; Deering et al., 1997; Glencross and Smith, 1997) *P. indicus* (Colvin, 1976; Read, 1981) *Litopenaeus vannamei* (Lim et al., 1997; Gonzalez-Felix et al., 2002a) (Gonzalez-Felix et al., 2002b) *P. japonicus* (Guary et al., 1976; Kanazawa et al., 1985) *P. indicus* (Colvin, 1976) and the freshwater prawn *Macrobrachium rosenbergii* (Sandifer and Joseph, 1976). Only a small number of studies have analysed feed related lipid changes in temperate lobster species. A few studies have investigated dietary and tissue lipids in the juvenile American lobster *Homarus americanus* (D'Abramo et al., 1980; Floreto et al., 2000; Floreto et al., 2001) and two studies are available on *Jasus edwardsii* larvae (Nelson et al., 2004) and adults (Smith et al., 2004).

Limited information on the lipid requirements of spiny lobsters exists. To formulate lobster feeds, it will be necessary to develop greater understanding of both lipid requirements and commercially available ingredients to meet those requirements. In addition, feed producers in the pig, poultry and aquaculture sectors are under increasing pressure to replace fish products in feeds as it is well recognised that fish meal and fish oil are a finite resource in decline (Rerat and Kaushik, 1995; Bell, 1998). The successful replacement of fish oil in aquafeeds will require identification of alternative oils that both promote growth and health during production with a final product that also meets market demands for providing nutritional value to humans. Oils are important in feeds for lobster not only to provide lipids and fatty acids, but also as phagostimulants, to provide pigments and vitamins. Potential oils sources include alternative marine oils rich

in omega 3 (n-3) polyunsaturated fatty acids (PUFA) generated from fisheries processing by-products e.g. salmon and tuna oil, or oil-rich marine meals that contribute significant quantities of endogenous lipids to the feed e.g. crustacean meals, squid meals, fish by-product waste. Vegetable oils are widely being examined as potential alternatives to marine lipids, as they are rich in C_{18} polyunsaturated fatty acids, and the monounsaturated fatty acid 18:1n-9c which may be preferentially catabolised for energy, and which has been observed to decrease dramatically in times of energy demand in *J. edwardsii* (Jeffs et al., 2002). Ingested lipids may be stored for energy reserves, catabolised immediately for energy or used in the biosynthesis of other lipids, hormones, steroids or cellular membranes. The fate of dietary lipids will depend on the amount of lipid ingested, the fatty acid composition and the energetic value of other dietary ingredients (D'Abramo, 1997).

Crustaceans obtain fatty acids for metabolic requirements through the absorption of ingested lipids and by biosynthesis of some fatty acids. Crustaceans are able to synthesize even-numbered carbon straight-chain saturated fatty acids (e.g. 16:0, 18:0, 22:0) *de novo* from acetate, and can convert these to monounsaturated forms (e.g. 16:1n-7, 18:1n-9) using the delta-9-desaturase system (D'Abramo, 1997), but have limited or no ability to synthesize the PUFA eicosapentaenoic acid (EPA, 20:5n-3) or docosahexaenoic acid (DHA, 22:6n-3). Crustaceans are unable to synthesize n-3 or n-6 fatty acids *de novo*, and cannot elongate and desaturate linoleic acid (18:2n-6) or linolenic acid (LNA,18:3n-3) to n-3 or n-6 highly unsaturated fatty acids (HUFA) (Bottino et al., 1980; Read, 1981). However, a study by (Kanazawa et al., 1979) did report limited ability to convert 18:3n-3 to 20:5n-3 and 22:6n-3. Castell and Boghen (1979) reported that juvenile *Homarus americanus* were unable to synthesize arachidonic acid (ARA, 20:4n-6), from dietary LNA as occurs in fish. ARA in fish tissues is almost exclusively positioned in the 2-position on the glycerol of phosphatidylinositol (PI) (Bell and Sargent, 2003). Given the importance of PI in crustaceans (Teshima, 1997), ARA has also been considered as essential (Castell and Boghen, 1979), in addition to the recognised essential fatty acids; linolenic acid, linoleic acid, EPA and DHA (Kanazawa and Koshio, 1994).

Cholesterol is also essential in the diet as unlike in mammals and teleosts, crustaceans cannot synthesise cholesterol from acetate or melanovate (Teshima, 1997; Kanazawa, 2001). Sterols are essential components of circulatory and membrane lipoproteins and are metabolic precursors to the formation of steroid and moulting hormones, essential for moulting (Cuzon and Guillaume, 2001) and growth. (Teshima, 1997; Cuzon and Guillaume, 2001). Diets deficient in sterols produced lower whole body lipid and serum cholesterol (D'Abramo et al., 1984). The inclusion of phospholipids, and in particular phosphatidylcholine (PC) and (PI) in *Homarus* spp. feeds has been shown to increase the transport of cholesterol to tissues (D'Abramo et al., 1982). PC and PI from soybean lecithin increase lipid transport and utilization in crustaceans and are recognized as beneficial for growth and survival of fish and crustacean larvae and juveniles. PC has been hypothesized to either mobilize ingested lipids through improved emulsification and absorption, or alternatively improve the movement of lipid (and subsequently cholesterol) from the digestive gland to the haemolymph and tissues (D'Abramo et al., 1985; D'Abramo, 1997). Presently both phospholipids (soybean lecithin) and cholesterol are recommended to be added to crustacean feeds until more definitive information as to their roles and requirements are determined.

The digestive gland is well recognized as the main lipid storage organ and its lipid content has been widely used as an index of nutritional status in crustaceans (Rosemark et al., 1980; Vogt et al., 1985; Al-Mohanna and Nott, 1987). Lipid reserves are required to fuel ecdysial processes, metabolism and reproductive development. Fatty acids obtained through biosynthesis or absorption provide an energy rich fuel that during optimum nutrition is stored in the digestive gland in the form of neutral lipid (triglyceride) droplets. Digestive gland lipid reserves have been shown to decrease during starvation preferentially before other energetic substrates (McLeod et al., 2004), and provide an energy reserve to compensate prolonged periods of non feeding in the wild. Fatty acids in muscle tissues generally occur in phospholipid membranes as structural components. The large abdominal tissues, may also provide an important lipid reserve once digestive gland lipid reserves are depleted during suboptimal nutrition, or starvation (McLeod et al., 2004). Changes in whole body lipid composition and content are generally more pronounced in larger lobsters than smaller lobsters (Dall, 1981; Cockcroft, 1997).

During periods of growth and optimal nutrition, the relative importance of fatty acids will differ from that during starvation (Jeffs et al., 2002). During growth, metabolism is geared toward protein synthesis and moulting, whereas during starvation the maintenance of essential structural lipids is more important. The utilization and importance of different lipids under high growth and optimal nutrition are not well understood. Dietary highly unsaturated fatty acids (HUFA's) are preferentially conserved in the polar lipid of body tissues in homarid lobsters (D'Abramo et al., 1980), *Macrobrachium rosenbergii* (D'Abramo and Sheen, 1993) and other penaeid species. D'Abramo (1997) suggested that unsaturated marine fish oils would be less likely to be stored for later use as energy, which may lead to potentially decreased levels in the digestive gland. It has been shown during starvation trials that larval *J. edwardsii* are able to retain or synthesize certain fatty acids preferentially (Smith et al., 2003b). Additionally the fatty acid profile of adult female *J. edwardsii* ovary is strictly controlled during development, where extruded egg lipid profiles remain of uniform composition independent of feeds, despite differing dietary lipid profile (Smith et al., 2004). Investigating the potential of different commercial ingredients (containing fatty acids) to promote rapid growth and ideal tissue composition under optimal growth conditions will improve current understanding of lipid nutrition of lobsters.

6.2.1 Aims

This study aims to observe the changes in fatty acid composition of lobsters fed formulated feeds made from commercial ingredients and where lipid content is contributed from a range of oil sources. The preferential retention of fatty acids and utilization for energy and or biosynthesis combined with growth response (Chapter 3), localised lipid histology (Chapter 4) and lipid class composition (Chapter 5) provide further understanding of the suitability of alternative lipid sources in lobster feeds.

6.3 Materials and Methods

6.3.1 General methods

Detailed general experimental and sampling methods are described in Chapter 3, (sections 3.3.1 to 3.3.12).This chapter focuses on the qualitative and quantitative fatty acid analysis of feeds, digestive gland and muscle tissue. The animals used in this chapter are from the experiment described in Chapter 3. A 10-week growth trial was conducted to assess the effects on growth and lipid tissue compositions of feeding juvenile lobsters *J. edwardsii* with 6 formulated feeds containing alternate lipid sources *ad libitum*. A 20-tank system was used with three tanks randomly allocated each of the formulated feeds, and the remaining two tanks were fed freshly opened blue mussels (*Mytilus edulis*). At the conclusion of the growth trial, growth measurements were recorded and digestive gland and the remaining tissues were dissected for fatty acid analysis.

6.3.2 Tissue sampling and dissection

At the conclusion of the trial all lobsters were dried and weighed, then the five most median sized lobsters in the intermoult stage (Turner, 1989) from each tank were killed in an ice-water slurry, and their digestive glands were immediately removed. The hindgut and stomach were removed from the digestive gland. Four of the digestive glands were bisected longitudinally; half was immediately frozen in liquid nitrogen for chemical and fatty acid analysis and half fixed for histology (Chapter 4). The remaining whole digestive gland was rapidly frozen in liquid nitrogen and halved for proximate and fatty acid analysis. The remaining whole body tissues (predominantly muscle tissues with exoskeleton) without the digestive gland were frozen in liquid nitrogen and is referred to as muscle tissue, MT throughout this chapter. The three muscle tissues selected for analysis included a sample from the initial population of lobsters, and the lobsters fed formulated feeds with the highest growth rates (MM) and the lowest growth rates (SQM). Two MT samples of 5 pooled lobsters were analysed in duplicate. Prior to chemical analyses, all tissues were freeze dried and homogenized using a mortar and pestle.

6.3.3 Fatty acid determination

Total lipid was determined gravimetrically in the initial extraction phase of fatty acid extraction using a modified Bligh and Dyer (1959) one-phase methanol:choloroform:water solvent system (2:1:0.8, by vol) for 24 h, after which the phases were separated by the addition of chloroform and water (final solvent ratio, 1:1:0.9, methanol:chloroform:water by vol). The lipid was concentrated by rotary evaporation at 40˚C. Lipid class analyses were performed immediately, with samples being stored for no more than 3 days in a known volume of chloroform (Sasaki and Capuzzo, 1984).

An aliquot of the total lipid extract was used to analyse the fatty acid composition. The lipid extract was transmethylated by reaction with methanol/hydrochloric acid/chloroform (10:1:1 v/v/v, 3 ml) for 1 h at 80°C. After cooling and addition of 1 ml, the resultant fatty acid methyl esters (FAME) then were extracted with hexane:chloroform (4:1 v/v 3 × 2 ml), and the FAME mixture was treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, 50 µl, 60°C) to convert sterols to their corresponding TMSi (trimethylsilyl) ethers. Analysis of fatty acid methyl esters were performed on a gas chromatograph (GC) (Hewlett Packard 5890A GC, Avondale, PA, USA) using a HP-5 cross-linked (5% Ph Me Silicone) capillary column (50 m × 0.32 mm × 0.17 film thickness), a flame ionisation detector, a split/splitless injector and an HP7673A auto sampler, with hydrogen as the carrier gas. Prior to analysis, samples were diluted with chloroform containing a known concentration of 19:0 or 23:0 FAME as the internal injection standard. Peaks were recorded and quantified using Waters Millenium 32 v3.05.01 (Milford, MA, USA). Component identification was determined by comparison with retention times and mass spectral data, and against authentic and laboratory standards as described previously (Bransden et al., 2005).

6.3.4 Statistical analysis and calculations

Concentrations of fatty acids and classes were presented on a qualitative (percentage of total lipid composition) and quantitative basis, where mg lipids were expressed per g wet tissue ($mg.g^{-1}$). Fatty acid analyses of samples of fresh mussel tissues gave results that were not typical fatty acid profiles of blue mussel in good condition, most likely due to the mussels spawning prior to sampling (Appendix 1). Good condition mussels were

used throughout the experiment and fatty acid profiles for good condition mussels were used from Murphy et al., (2000) who used identical laboratory procedures and blue mussels sampled from the same region in Tasmania (P.D. Nichols, personal communication).

Homogeneity of variance was assessed by examining data residual plots. Where tissues and feed compositions were compared, one-way analysis of variance (ANOVA) was used. Significance was accepted where $P<0.05$. Statistical analyses were performed using SPSS 11.5.0. All analyses were performed in duplicate on a pooled sample of half-digestive glands from five individual lobsters from each tank. Data is reported as mean ± standard deviation (S.D.) from two replicate tanks (n=2) unless stated otherwise.

6.4 Results

Of the 57 fatty acids present in feeds and body tissues, the four major fatty acids present in all samples were palmitic acid (16:0), oleic acid (cis-isomer) 18:1n-9c, eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA).

6.4.1 Feed fatty acid composition

The four main fatty acids EPA, DHA, 18:1n-9c, and 16:0 made up approximately 50% (FOL and MM) to 66% (CO) of the total fatty acids. CO contained the highest composition of 18:1n-9c (43%), and FrM the least (2%). Levels of 16:0 were similar in the marine based feeds FrM, MM, SQM, TO and FO (19-21%), but were lower in the plant lipid feeds FOL (16%) and CO (11%) (Table 6.1). FrM, TO and FO contained high levels of DHA (21%, 20% and 14%, respectively) compared to all other feeds (2-9%). The plant based feeds FOL and CO contained the highest proportions of linoleic acid (18:2n-6) (25% and 10% respectively) (Fig. 1). The fatty acid content of the feeds (mg.g DM^{-1}) (Table 6.2) followed similar trends as the percentage fatty acid content (Table 6.1).

The proportion of saturated fatty acids (SFA) in the feeds was lowest in CO (19%), whereas FOL (25%) and SQM (36%) contained significantly higher proportions of SFA than all other feeds ($F_{6,8}$=43.5, P<0.001) (Table 6.3). Monounsaturated fatty acids (MUFA) were inversely related to SFA where CO had the highest percentage (52%) and FrM the lowest (11%) percentage of MUFA ($F_{6,8}$=289.2, P<0.001). Polyunsaturated fatty acids (PUFA) were significantly lower in CO, SQM and MM (25-32%), and PUFA were in significantly higher proportions in FOL (47%) and FrM (48%) than all other feeds except FO (40%) ($F_{6,8}$=37.7, P<0.001). The total proportion of n-3 fatty acids was highest FrM (41.5%) and significantly lower in plant based feeds CO, FOL and SQM (14-19%) ($F_{6,8}$=54.1, P<0.001). Conversely, the proportion of n-6 fatty acids was highest in FOL (27%) and CO (10.7%), with no difference between the rest of the marine based feeds (4-6%) ($F_{6,8}$=84.4, P<0.001). Ratios between the sum of n-3/n-6 fatty acids indicated significant differences existed between all feeds (Table 6.4.) and FrM and FO had significantly higher ratios (8.5) than the other feeds (3-6), and where FOL and CO had the lowest n-3/n-6 ratios (<1.5) ($F_{6,8}$=25.7, P<0.001). Ratios between the essential PUFA, EPA:ARA, were significantly higher in FrM (95.2) than all other feeds ($F_{6,8}$=10.6, P=0.002). DHA:EPA ratios were lowest in MM (0.7) and highest in TO (2.3) ($F_{6,8}$=134.4, P<0.001) (Table 6.4).

6.4.2 Feed sterol composition

Sterol content in feeds was dominated by cholesterol, with stigmasterol present in all feeds except SQM and TO (Table 6.5). FrM, MM and FO contained the widest variety of sterols (Table 6.5 & 6.6), and FrM had consistently higher relative levels of each sterol than the formulated feeds. Vitamin A was present in all feeds except TO and SQM (Table 6.5).

6.4.3 Digestive gland fatty acid - percentage lipid composition

The fatty acid composition of the digestive gland tissue (Table 6.7) closely reflected the fatty acid composition of the feeds (Fig.1). The proportion of the total fatty acids made up by the four main fatty acids (16:0, 18:1n-9c, DHA, EPA) ranged from 49% (CO) to 66% (MM). The initial lobsters were within this range, where the four main fatty acids comprised 54% of the total fatty acids.

There was no significant difference in digestive gland proportions of SFA ($F_{7,8}$=2.6, P=0.107) or MUFA ($F_{7,8}$=1.5, P=0.302) as percentage of total fatty acids (Table 6.8). However, there were significant differences in the proportions of PUFA where FrM (26%) was significantly lower than TO, FOL and the initial lobsters, and where FOL had significantly higher levels of PUFA than MM, CO, FO, SQM ($F_{7,8}$=11.5, P=0.001) (Table 6.8). The total proportion of n-3 fatty acids in digestive glands of lobsters was not significantly different between any of the feeds or the initial lobsters ($F_{7,8}$=3.5, P=0.051). However, there were marked differences in the n-6 proportions of lobster digestive glands where FO and MM (5%) had significantly lower n-6 fatty acids than the initial lobster population (13%), and FOL (13%) ($F_{7,8}$=28.85, P<0.001) (Table 6.8). The n-3/n-6 ratio in the digestive glands also showed differences according to feed, where FOL (1) was significantly lower than FO (7) ($F_{7,8}$=3.8, P=0.006). The EPA/ARA ratio was significantly lower in FrM (2) compared to FOL, CO, FO and MM (8-9) ($F_{7,8}$=7.4, P=0.006) and there were no significant differences in the DHA/EPA ratios in digestive glands of lobsters fed any of the feeds (Table 6.8).

6.4.4 Digestive gland fatty acid - quantitative composition

The four main fatty acids in the digestive gland made up the largest proportion by weight in the FrM fed lobsters (52 $mg.g^{-1}$) (Fig 2). Similar proportions were seen between CO (49 $mg.g^{-1}$) fed lobsters, MM (47 $mg.g^{-1}$) and FOL (44 $mg.g^{-1}$), while that level was halved in TO (22 $mg.g^{-1}$), FO (20 $mg.g^{-1}$) and SQM was even lower at 13 $mg.g^{-1}$ (Table 6.9).

When the total weight of each fatty acid per gram of wet tissue ($mg.g^{-1}$) was assessed, substantial differences were evident in digestive gland fatty acid contents between lobsters fed the different feeds, and those of the reference feed (FrM) fed and initial lobster populations (Table 6.9 & 6.10). The digestive glands of lobsters fed the FrM and MM (38-40 $mg.g^{-1}$) had similar SFA content to the initial lobsters and significantly higher SFA content than lobsters fed all other feeds, and the lowest SFA content occurred in lobsters fed SQM (5.4 $mg.g^{-1}$) ($F_{7,8}$=26.7, P<0.001) (Table 6.10). MUFA digestive gland fatty acid contents were highest in the initial lobsters and CO, MM and FrM (36-45 $mg.g^{-1}$) and ranged down to 8.8 $mg.g^{-1}$ in the SQM fed lobsters ($F_{7,8}$=29.5, P=0.001) (Table 6.10). Digestive gland PUFA contents were similar between initial

lobsters and FrM, FOL (36-42 $mg.g^{-1}$) and lowest in the FO, TO and SQM fed lobsters (8-14 $mg.g^{-1}$) (Fig 3A). The total amount of n-3 fatty acids in digestive glands was similar between the initial lobsters and lobsters fed all feeds except FO and SQM fed lobsters (6-10 $mg.g^{-1}$) ($F_{7,8}$=7.3, P=0.006). The total amount of all digestive gland n-6 fatty acids was highest in lobsters fed FOL (23 $mg.g^{-1}$) and the initial lobsters (13 $mg.g^{-1}$) ($F_{7,8}$=46.3, P<0.001). The ratio of digestive gland n-3 /n-6 fatty acids was similar between initial lobsters and those fed most feeds (2-5), however FO (7) was significantly higher than the FOL (1) ($F_{7,8}$=3.9, P=0.038). Digestive gland ratios of EPA/ARA were similar between all lobsters fed formulated feeds (4-9), but significantly lower in the FrM fed lobsters (2) ($F_{7,8}$= 7.4, P=0.006) (Fig. 3B). The DHA/EPA ratio was highest in the CO and FOL treatments (10) and similar between lobsters fed all feeds except SQM and FrM, and the initial lobsters (3-5) ($F_{7,8}$= 9.7, P=0.02) (Table 6.10).

6.4.5 Muscle tissue fatty acid - percentage composition

The four main fatty acids present in muscle tissues from the initial population of lobsters and lobsters fed the formulated feeds MM and SQM comprised 58% of the total fatty acids in initial lobsters, and between 59% and 63% (MM and SQM fed lobsters, respectively). Fatty acid compositions of the muscle tissues were similar between the three feeds assessed (Table 6.11), and did not show as much variation in proportions of fatty acids as digestive gland tissues (Fig. 1). The initial lobsters had higher 18:2n-6 and ARA with lower EPA and 20:3n-6 levels than MM and SQM fed lobsters. MM fed lobsters had higher muscle tissue 18:0, but lower DHA content than SQM and the initial lobsters. After feeding on SQM, lobster muscle tissues contained higher proportions of 18:1n-9c and 18:1n-7c (Fig. 1).

SFA were slightly different between the initial lobsters (30%) and SQM fed lobsters (28%), with MM fed lobster muscle tissue containing higher SFA levels (32%) ($F_{2,3}$=32.1, P=0.009) (Table 6.12). MUFA were significantly different between all feeds ($F_{2,3}$=45.7, P=0.06), and were higher by proportion in SQM (32%) than MM (28%) and initial lobsters (25%) ($F_{2,3}$=45.7, P=0.006). PUFA were highest in the muscle tissue of the initial lobsters (42%) and significantly lower in MM (37%) and SQM (38%)

($F_{2,3}$=16.2, P=0.025) (Fig 3A. The n-3/n-6 ratio was markedly higher in MM (6.0) fed lobster muscle tissues than in SQM fed (4.3) or initial lobsters (2.7) ($F_{2,3}$=239.0, P<0.001). EPA/ARA ratios were significantly different with MM (7.5) higher than the SQM (5.0) or initial (2.9) in the muscle tissues ($F_{2,3}$=1477.6, P<0.001) (Fig. 3B). DHA/EPA ratios also differed significantly between each of the three muscle tissues, where MM (0.7) was lower than SQM (0.8) and the initial lobsters (1.0) ($F_{2,3}$=126.2, P=0.001) (Table 6.12).

6.4.6 Muscle tissue fatty acid - quantitative composition

The content of fatty acid classes in the muscle tissues varied significantly between the three treatments analysed (Table 6.13). Total SFA, MUFA and PUFA and total n-3 fatty acids were all significantly higher in the muscle tissues of lobsters fed MM, than the initial lobsters, while SQM fed lobsters had consistently lower content of SFA, MUFA, and PUFA than the initial and MM fed lobsters ($F_{2,3}$=1822.0, P<0.001) (Fig. 4A). The total n-6 fatty acids were highest in the initial population of lobsters (11) while SQM (7) was significantly higher than MM fed lobster muscle tissues (5) ($F_{2,3}$=473.8, P<0.001) (Table 6.14). Similarly the n-3/n-6 ratio was highest in MM fed lobsters, whereas SQM fed lobsters had a higher muscle tissue ratio (5) than the initial lobsters (3) ($F_{2,3}$=239.0, P<0.001). The ratio of EPA/ARA differed between all feeds and was highest in MM fed lobsters (8), SQM (5) and lowest in the initial lobsters (3) (Table 6.14). DHA/EPA ratios were high in the initial population, while SQM (0.8) and MM (0.7) were not notably different (Fig. 4B)

6.5 Discussion

6.5.1 Feed composition

The range of formulated feeds showed markedly different fatty acid compositions. The feed composition was influenced mainly by the terrestrial or marine origin of the lipid. The plant oils (soybean lecithin and canola oil) contributed higher levels of 18:2n-6 and 18:1n-9c, lower proportions of n-3 and lower essential fatty acids DHA and EPA than other marine oil and meal based feeds. The SQM and MM feed contained high levels of

SFA (particularly 14:0, 15:0, 18:0), which has been observed in other squid meal containing feeds (Merican and Shim, 1994). Oil from the high order carnivore, tuna, contains high levels of PUFA, in particular DHA and accordingly has been included in experimental feeds specifically to increase DHA levels (Bransden et al., 2005). Elevated DHA resulted in the TO feed. FrM and MM feed and lobster tissues contained a wider variety of minor fatty acids (Tables 1, 5 and 7) likely as a result of microalgal filter feeding (Bottino et al., 1980).

6.5.2 Initial lobsters

Several studies have analysed the fatty acid composition of wild adult *J. edwardsii* (Nichols et al., 1998), wild puerulus (Jeffs et al., 2002) and both wild (Pearce, 1997) and cultured phyllosoma (Smith et al., 2003a; Smith et al., 2004). Early juvenile lobsters however, are difficult to find due to their cryptic nature and no studies have reported fatty acid compositions of wild juveniles. Post puerulus can be caught easily using collectors as they become benthic after the extended larval stages (Mills and Crear, 2004). Studies have shown post puerulus typically have depleted lipid reserves and very little triglyceride content after their extended inshore migration, the last stages of which are non-feeding (Jeffs et al., 2001). The fatty acid profiles and EFA ratios of the initial animals was typical of other reports with the main 4 fatty acids in muscle tissues at very similar levels to whole body compositions of wild caught *J. edwardsii* post-puerulus (Pearce, 1997; Jeffs et al., 2004) newly hatched phyllosoma (Smith et al., 2003b), and adults (Nichols et al., 1998). Levels of EPA and ARA were higher in the initial lobsters than those fed formulated feeds, and similar to the lobster fed the reference feed FrM.

6.5.3 Relationships between tissue fatty acid profile and growth

Compared to the initial lobsters that were sampled prior to the experiment, there were marked differences in the percentage fatty acid profiles and EFA ratios between digestive gland and muscle tissue compositions that were closely related to the feed. The patterns in qualitative composition did not clearly describe differences related to growth from Chapter 3. The percentage fatty acid profile of lobsters with lowest digestive gland lipid accumulation and slowest growth (SQM), most closely resembled the fatty acid profile of muscle tissues. The selective conservation of essential fatty acids within structural phospholipids membranes, and catabolism of the majority of non-essential

lipids (Dall, 1981; Jeffs et al., 2002) suggests that neutral lipid storage in the SQM treatment was minimal, as confirmed by histology (Chapter 4). The differing rates in quantitative accumulation of the main four fatty acids (16:0, 18:1n-9c, DHA and EPA) in the digestive gland generally resembled the patterns seen in growth (Chapter 3) and lipid storage as lipid droplets within R-cells of the digestive gland (Chapter 4). Jeffs et al., (2002) have shown energy substrates in the non-feeding puerulus stages of *J. verreauxi* to include 16:0, 18:0, 18:1n-9c, 16:1n-7c. The proportions of these fatty acids were markedly decreased in the FO, TO and SQM fed lobsters which were also the feeds that showed the least lipid droplet storage (Chapter 4). Tuna oil in feeds for juvenile *Homarus* spp. was shown to decrease depot fat levels in the digestive gland (D'Abramo et al., 1980). Jeffs (2002) suggested selective catabolism occurred in *J. edwardsii* puerulus where PUFA and the essential fatty acids EPA, DHA, ARA and LNA were conserved and SFA and MUFA catabolised. Kanazawa and Koshio (1994) showed that EPA and DHA were incorporated at higher concentrations (76-91%) in the phospholipids than in the neutral lipids (9-24%). The present study showed higher concentrations of DHA and EPA in the muscle tissues, in which phospholipids are the dominant lipid class (Chandumpai et al., 1991). The slowest growing lobsters (SQM) in the present study also showed significant depletions in each of the essential fatty acids, although to a lesser degree than that of SFA and MUFA. Decreases in EPA were observed in adult *J. edwardsii* fed squid-pellet and beef-pellet combination feeds (Smith et al., 2004). In the present study, SQM fed lobsters were shown to have significantly lower whole body protein (Chapter 3, Table 6.3) indicating that proteins in muscle tissues were being catabolised. It is well recognized that the efficiency with which a lipid source will be utilized is dependent on the lipid quality, protein quantity and quality, and availability of other energy sources in the feed (D'Abramo, 1997). Although the feed intake was not different between all feeds, it is possible that some unknown factor influenced the poor lipid deposition of SQM fed lobsters.

In the present study the fastest growing lobsters fed FrM had similar digestive gland EFA ratios (qualitative and quantitative) to the slowest growing lobsters fed SQM. The ratios of essential fatty acids (EFA) affect the composition of feeds and muscle tissue compositions, but did not directly affect growth rates, which is supported by a previous study in *H. americanus* (D'Abramo et al., 1980). Gerring (1992) found no differences in

growth rates in *J. edwardsii* fed coconut oil, canola oil and cod liver oil. Similarly, D'Abramo et al., (1980) showed that *H. americanus* fed purified feeds containing tuna oil, corn oil and cod liver oil also had similar growth rates. In *P. japonicus*, n-3 fatty acids produced faster growth than n-6 fatty acids (Sandifer and Joseph, 1976; Kanazawa et al., 1977) and ratios of n-3/n-6 of 3.1 to 1.1 have produced good growth in *Macrobrachium rosenbergii* (Sandifer and Joseph, 1976). In contrast, Guary et al (1976) reported an increase in n-6 fatty acids and an associated decrease in growth in *P. japonicus* fed a feed containing soybean oil. The present study shows that for *J. edwardsii* the use of non-marine fatty acids in feeds has no detrimental effects to growth performance.

6.5.4 Relationships between dietary and tissue fatty acid composition

In the present study, the digestive gland percentage fatty acid composition of juvenile lobsters was closely related to the dietary fatty acid composition as has been demonstrated in adult *J. edwardsii* (Smith et al., 2004) and *H. americanus* (D'Abramo et al., 1980) (Fig. 1). The most abundant fatty acid in digestive gland tissues of MM and FrM and initial lobster was 16:0, which is similar to previous studies of wild postlarval *J. edwardsii* (Pearce, 1997). The EPA/ARA ratio supplied in the feeds FOL, FO, SQM, MM and FrM, was higher than in the digestive gland, while CO and TO maintained similar ratios to that in the feed (Fig. 3). The DHA/EPA ratio was higher in feeds FrM, TO and SQM than in the corresponding digestive glands (Fig. 4). The digestive glands of formulated feed fed lobsters in the present study had lower DHA/EPA ratios (0.8 to 1.4) than those of wild phyllosoma (1.8 to 4.6) (Phleger et al., 2001). Smith et al., (2004) reported DHA/EPA ratios of (1.4 to 1.8) for lobsters fed a beef heart/pellet feed and (2.3 to 3.3) when feed a squid meal pellet. As a proportion of total lipid, there were no differences in the individual concentrations of the EFA, DHA and EPA. D'Abramo (1980) showed a higher ratio of neutral/polar lipids in the digestive glands of marine oil fed lobsters, where DHA and EPA were conserved in the polar lipid fraction. In the present study, fatty acid compositions of separate lipid fractions were not measured, however the muscle tissue contained a higher percentage of DHA and EPA than the digestive gland tissues in all treatments. The fresh mussel fed lobsters were the least similar in percentage fatty acid profile to their feed. However, it is important to note that the fresh mussel data was derived from Murphy et al., (2002), and variation in fatty

acids profile may occur with seasonal variation in algal species composition (Jeffs et al., 2004) and mussel reproductive condition at the time of analysis.

The percentage fatty acid composition of muscle tissue varied only between the feeds, as was observed in adult lobster muscle tissue (Smith et al., 2004), and is consistent with the structural role lipids play in muscle tissues (Dall, 1981). However, on a quantitative basis the MM fed lobsters had consistently higher concentrations of each fatty acid in their muscle tissues than the SQM fed and initial lobsters, except ARA, which was higher in the initial lobsters (Table 6.11). The most abundant fatty acids ($mg.g^{-1}$) in the muscle tissues varied between the three samples; MM (16:0 and EPA), SQM (18:1n-9c) and 16:0 for initial lobsters. Pearce, (1997) found 16:0 to dominate whole body tissues of wild caught postlarval *J. edwardsii*. SQM contained the highest proportion of 18:1n-9c in muscle tissue, and among the highest digestive gland concentrations, whereas lobsters with the highest growth rates (FrM and MM) contained relatively lower proportions.

6.5.5 Implications for future feed formulation

The requirement for cholesterol has not been demonstrated in *J. edwardsii*. However, mortality due to incomplete ecdysis, similar to the moult death syndrome described in homarid lobsters (Bowser and Rosemark, 1981), has been observed in juvenile *J. edwardsii* (Ward, unpublished data; Gerring, 1992). The addition of soybean lecithin in *J. edwardsii* feeds in a previous study reduced the occurrence of MDS (Gerring, 1992), suggesting a similar requirement for phospholipid in *J. edwardsii* juveniles as occurs for other juvenile lobsters (Conklin et al., 1980; Kanazawa and Koshio, 1994). It would appear that the supply of cholesterol, phospholipids and ARA are necessary in the feeds of spiny lobsters and the lipid sources used in commercial feed applications must include a dietary source of these nutrients. The good growth response of lobsters fed CO and FOL feeds suggests promising potential for the inclusion of non-marine oils in feeds for lobsters. The inclusion of lecithin shows potential to increase the lipid levels of the digestive gland as seen in *P. japonicus* (Kontara, 1997), however we need more definitive information to further understand the implications of high lipid levels in rock lobster digestive glands. In slow growing lobsters, high digestive gland lipid may present an malfunction in lipid mobilization causing, or related to, a pathological

condition similar to fatty liver syndrome (D'Abramo et al., 1980; Tsvetnenko et al., 2000). In fast growing lobsters, high digestive gland lipids are recognised as a vital energy reserve in preparation for moulting and growth, and in *Jasus lalandii* has been directly related to growth increment at moult (Cockcroft, 1997). It is possible that both situations may occur, depending on the composition and nutritive value of the oil stored.

When compared to FO, the inclusion of soybean lecithin in the FOL feed did not appear to change the proportions of lipid which were utilized from the feed. Both treatments had similar feed and tissue compositions. However, on a quantitative basis, there was an increased quantity of lipid in FOL fed lobster digestive gland tissues compared to the FO fed lobsters. Whether this disparity is effected by lecithin based phospholipids increasing the mobilization of ingested lipids to the digestive gland resulting in high digestive gland lipid values, or whether the high lipid levels are due decreasing mobilization from the digestive gland to the tissues, remains in question (D'Abramo et al., 1985; D'Abramo, 1997). More nutritive lipid sources have been shown to increase the total lipid level in the digestive gland and tissues. While the total lipid gained during the growth experiment was shown to be positively related to growth ($r^2 = 0.76$, $F=37.5$, $P<0.001$) as previously seen in *J. edwardsii* (Johnston et al., 2003; Ward et al., 2003), the major fatty acids in Fig. 2, did not explain the differences in growth observed. For example, TO and FO, both showed lower digestive gland total lipid levels, but also good growth. This same issue was demonstrated in Chapter 3, where TO and FO had higher LER, but MM and FOL had higher PLV.

6.6 Conclusion

There was no detrimental effect of feeding CO and FOL based feeds to lobsters where they received a low level of essential fatty acids from the marine meals. While there were differences in the fatty acid composition of the muscle tissue and digestive gland (Fig. 2), the n-3/n-6 ratios were not markedly different in the digestive gland (Table 6.10) or muscle tissues (Table 6.14), which is of concern to maintain health benefits and product acceptability for human consumers (Nichols et al., 1998). Decreases in lipid retention in the digestive gland in lobsters that grew slowly could not be directly

attributed to a deficiency or imbalance of fatty acids. The qualitative and quantitative fatty acid profiles of digestive gland tissues were strongly influenced by the fatty acid composition of the feed, while muscle tissues changed in total lipid content, but not percentage fatty acid profile. FrM promoted the most similar lipid compositions to the initial lobsters, and the best growth rates. SQM produced poor growth, and an advanced depletion of both non-essential fatty acids and essential fatty acids in the digestive gland and the muscle tissues.

Table 6.1. Dietary fatty acid percentage composition (%) (mean ± S.D.) of feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and the reference feed fresh mussel (FrM) fed to juvenile lobsters *Jasus edwardsii* over a 10 week growth trial.

	CO	FOL	FO	TO	SQM	MM	FrM[2]
	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.
14:0	2.1 ± 0.0	3.0 ± 0.3	5.1 ± 0.2	3.5 ± 0.3	4.4 ± 0.0	6.4 ± 0.6	1.3 ± 0.6
i15:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	n.d.
a15:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	n.d.
15:0	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	2.3 ± 2.7	0.7 ± 0.0	0.2 ± 0.1
i16:0	0.5 ± 0.0	0.4 ± 0.6	0.9 ± 1.1	0.6 ± 0.0	1.0 ± 0.1	1.2 ± 0.1	n.d
C16PUFA	0.4 ± 0.0	1.0 ± 0.6	0.7 ± 0.9	0.3 ± 0.0	0.9 ± 0.0	1.2 ± 0.2	n.d
16:1n-9c	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.1
16:1n-7c	2.5 ± 0.0	3.5 ± 0.0	6.8 ± 0.3	4.9 ± 0.3	6.5 ± 0.0	8.6 ± 0.2	3.3 ± 2.0
16:1n-5c	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	n.d
16:0	11.0 ± 0.2	15.5 ± 0.4	18.7 ± 1.1	18.9 ± 1.0	20.9 ± 0.0	20.3 ± 0.3	20.6 ± 2.0
16:0 Fade	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.7 ± 0.2
i17:0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.4 ± 0.1	0.7 ± 0.1
a17:0	0.2 ± 0.2	0.2 ± 0.0	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.0	0.2 ± 0.0	n.d
17:1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	n.d
17:1n-8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	n.d
17:0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.3	0.9 ± 0.0	0.7 ± 0.0	1.0 ± 0.0	n.d
18:3n-6	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.3 ± 0.3	0.1 ± 0.0	0.1 ± 0.1	0.6 ± 1.8
18:4n-3	0.8 ± 0.0	1.3 ± 0.0	2.8 ± 0.3	1.3 ± 0.0	1.8 ± 0.1	2.6 ± 0.0	1.6 ± 0.9
18:2n-6	9.7 ± 3.1	25.2 ± 1.0	2.1 ± 0.2	1.9 ± 0.0	4.0 ± 0.2	2.0 ± 0.0	1.1 ± 0.2
18:1n-9c[4]	43.0 ± 1.2	17.2 ± 0.8	10.7 ± 0.8	12.1 ± 0.3	19.2 ± 2.0	8.6 ± 0.1	1.9 ± 0.9
18:1n-7c	3.4 ± 0.0	2.4 ± 0.1	3.3 ± 0.2	2.8 ± 0.1	2.1 ± 2.5	3.3 ± 0.1	2.0 ± 0.2
18:1(n-7)t	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.1 ± 0.0
18:0	3.1 ± 0.1	4.1 ± 0.2	4.3 ± 0.4	4.9 ± 0.1	5.1 ± 0.3	4.4 ± 0.1	4.4 ± 1.1
18:0 FAde	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.6 ± 0.3	n.d
20:4n-6	0.6 ± 0.0	0.7 ± 0.0	1.2 ± 0.1	1.7 ± 0.1	0.6 ± 0.0	0.9 ± 0.0	1.7 ± 0.4
20:5n-3	5.3 ± 0.5	7.8 ± 0.2	15.2 ± 1.3	9.0 ± 0.4	8.2 ± 0.6	12.4 ± 0.4	14.6 ± 2.1
20:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	0.4 ± 0.1
20:4n-3	0.3 ± 0.0	0.4 ± 0.0	0.9 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.7 ± 0.0	2.1 ± 0.6
20:2n-6	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.8 ± 0.1	0.2 ± 0.0	0.7 ± 0.1

20:1(n-9)c	1.1 ± 0.0	0.6 ± 0.0	1.1 ± 0.0	1.6 ± 0.0	1.3 ± 0.1	2.2 ± 0.1	1.2 ± 1.5
20:1(n-7)c	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.9 ± 0.0	0.8 ± 0.1
20:0	0.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	n.d
21:5n-3	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.4	0.3 ± 0.0	0.3 ± 0.1	0.5 ± 0.0	0.4 ± 0.1
22:5n-6	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	1.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	1.1 ± 0.1
22:6n-3	6.6 ± 0.8	8.6 ± 0.2	13.6 ± 0.2	20.4 ± 1.5	6.7 ± 0.8	8.4 ± 0.7	21.2 ± 3.9
22:4n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
22:5n-3	0.9 ± 0.1	1.2 ± 0.0	2.2 ± 0.1	1.7 ± 0.2	1.1 ± 0.2	1.5 ± 0.1	1.1 ± 0.1
22:1(n-11)	0.2 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	1.1 ± 0.2	0.7 ± 0.1	n.d
22:1(n-9)	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	n.d
22:1(n-7)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.3 ± 0.4	0.1 ± 0.0	n.d
22:2NMI	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.2	2.6 ± 0.5
22:0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.3	0.4 ± 0.3	0.1 ± 0.1	n d
Others[1]	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.3	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	7.13[3] ± 0.6

n.d., not detectable

[1] Others ıncludes fatty acids with less than 0.2%; 16:1n-7t, 17:1n-6, i18:0, 18:1n-5c, i19:0, 19:1, 20:1n-11c, C21 PUFA C22 PUFA, 24:1, 24:0

[2] Fresh mussel fatty acid composition from Murphy et al., 2002.

[3] Others calculated from mean site data (n=3) in (Murphy et al., 2002), includes 17:2, C_{22} PUFA, Dimethylacetal (DMA) of 16:0 and 18:0 and 18:0 fatty aldehyde, 28:8n-3, C_{23}PUFA, 20:1n-11c, 20:2 NMI, 19:1, 18:5n-3, i18:0, 16:1n-7t, 4,8,12 trimethyl tetradecanoic acid (TMTD).

[4] Includes 18:3n-3

Table 6.2. Quantitative dietary fatty acid (FA) composition (mg FA.g pellet DM^{-1}) (mean ± S.D.) of feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and of the reference feed fresh mussel (FrM).

	CO D		FOL D		FO D		TO D		SQM D		MM D		FrM[1]	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
14:0	2.5	0.0	3.8	0.4	6.3	0.3	4.2	0.4	7.3	0.1	9.9	0.9	0.9	0.1
i15:0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.2	0.1	0.3	0.0	0.0	0.0
a15:0	0.1	0.0	0.1	0.0	0.2	0.1	0.0	0.0	0.1	0.1	0.2	0.0	0.0	0.0
15:0	0.2	0.0	0.3	0.0	0.6	0.1	0.8	0.1	3.9	4.4	1.1	0.0	0.3	0.1
i16:0	0.6	0.0	0.6	0.8	1.1	1.3	0.8	0.0	1.7	0.1	1.9	0.2	0.0	0.0
C16PUFA	0.5	0.0	1.3	0.8	0.8	1.1	0.4	0.0	1.4	0.0	1.9	0.2	0.0	0.0
16:1n-9c	0.2	0.0	0.1	0.0	0.3	0.0	0.3	0.0	0.4	0.0	0.3	0.0	0.1	0.0
16:1n-7c	3.0	0.1	4.6	0.0	8.3	0.4	6.0	0.3	10.6	0.0	13.4	0.3	2.3	0.6
16:1n-5c	0.1	0.0	0.1	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.3	0.0	0.0	0.0
16:0	12.9	0.2	20.1	0.5	23.0	1.3	23.1	1.2	34.3	0.0	31.5	0.5	14.0	0.0
16:0 Fade	0.0	0.0	0.0	0.0	0.1	0.2	0.5	0.0	0.2	0.0	0.1	0.2	0.5	0.1
i17:0	0.1	0.0	0.1	0.0	0.2	0.2	0.2	0.0	0.4	0.2	0.6	0.1	0.5	0.0
a17:0	0.2	0.3	0.2	0.0	0.2	0.1	0.3	0.3	0.2	0.1	0.2	0.0	0.0	0.0
17:1	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17:1n-8	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.1	0.4	0.0	0.0	0.0	0.0	0.0
17:0	0.4	0.1	0.6	0.0	0.6	0.4	1.2	0.0	1.2	0.0	1.6	0.0	0.5	0.1
18:3n-6	0.1	0.0	0.1	0.0	0.1	0.1	0.4	0.3	0.2	0.0	0.2	0.1	0.4	0.7
18:4n-3	0.9	0.1	1.7	0.0	3.5	0.3	1.6	0.0	3.0	0.2	4.1	0.0	1.2	0.4
18:2n-6	11.4	3.7	32.8	1.3	2.5	0.2	2.3	0.0	6.6	0.3	3.1	0.0	0.8	0.1
18:1n-9c[4]	50.5	1.5	22.4	1.0	13.1	1.0	14.9	0.4	31.5	3.3	13.3	0.2	1.3	0.5
18:1n-7c	3.9	0.0	3.2	0.1	4.1	0.3	3.4	0.1	3.5	4.1	5.1	0.1	1.4	0.1
18:1(n-7)t	0.2	0.0	0.3	0.0	0.5	0.1	0.3	0.0	1.0	0.0	0.6	0.0	0.1	0.0
18:0	3.7	0.1	5.4	0.3	5.3	0.4	6.0	0.2	8.4	0.4	6.8	0.1	3.1	0.5
18:0 FAde	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.2	1.0	0.5	0.0	0.0
20:4n-6	0.7	0.1	0.9	0.0	1.4	0.1	2.1	0.1	1.1	0.1	1.4	0.0	1.2	0.1
20:5n-3	6.3	0.5	10.2	0.3	18.7	1.6	11.0	0.5	13.5	1.0	19.3	0.7	10.2	0.8
20:3n-6	0.1	0.0	0.1	0.0	0.2	0.1	0.1	0.0	0.1	0.0	0.9	0.0	0.3	0.1
20:2NMI	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.3	0.1

20:4n-3	0.3 ± 0.0	0.5 ± 0.0	1.1 ± 0.2	0.6 ± 0.0	0.9 ± 0.1	1.1 ± 0.0	1.5 ± 0.3
20:2n-6	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	1.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.0
20:1(n-9)c	1.3 ± 0.1	0.8 ± 0.0	1.4 ± 0.0	2.0 ± 0.0	2.1 ± 0.2	3.4 ± 0.1	0.9 ± 0.3
20:1(n-7)c	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.4 ± 0.0	1.3 ± 0.1	0.6 ± 0.0
20:0	0.6 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	1.1 ± 0.1	0.3 ± 0.0	0.0 ± 0.0
21:5n-3	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.5	0.4 ± 0.0	0.4 ± 0.1	0.8 ± 0.0	0.1 ± 0.0
22:5n-6	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	1.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.1 ± 0.0
22:6n-3	7.8 ± 0.9	11.2 ± 0.2	16.7 ± 0.3	25.0 ± 1.8	11.1 ± 1.3	13.0 ± 1.0	14.8 ± 2.1
22:4n-6	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
22:5n-3	1.0 ± 0.1	1.6 ± 0.0	2.7 ± 0.1	2.1 ± 0.2	1.8 ± 0.3	2.3 ± 0.1	0.8 ± 0.0
22:1(n-11)	0.3 ± 0.0	0.5 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	1.8 ± 0.4	1.1 ± 0.1	0.0 ± 0.0
22:1(n-9)	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	1.0 ± 0.1	0.3 ± 0.0	0.0 ± 0.0
22:1(n-7)	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.6 ± 0.6	0.1 ± 0.0	0.0 ± 0.0
22:2NMI	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.3	0.9 ± 0.1
22:0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.5 ± 0.4	0.6 ± 0.5	0.1 ± 0.1	0.0 ± 0.0
Others[2]	0.1 ± 0.0	0.0 ± 0.0	0.4 ± 0.3	0.1 ± 0.0	0.0 ± 0.1	0.2 ± 0.0	2.3 ± 0.1

n.d., not detectable

[1]Others includes fatty acids with less than 0.2%; 16:1n-7t, 17:1n-6, i18:0, 18:1n-5c, i19:0, 19:1, 20:1n-11c, C21 PUFA C22 PUFA, 24:1, 24:0

[2] Fresh mussel fatty acid composition from Murphy et al., 2002.

[3] Others calculated from mean site data (n=3) in (Murphy et al., 2002), includes 17:2, C_{22} PUFA, Dimethylacetal (DMA) of 16:0 and 18:0 and 18:0 fatty aldehyde, 28:8n-3, C_{23}PUFA, 20:1n-11c, 20:2 NMI, 19:1, 18:5n-3, i18:0, 16:1n-7t, 4,8,12 trimethyl tetradecanoic acid (TMTD).

[4] Includes 18:3n-3

Table 6.3. Percentage fatty acid classes and essential fatty acid ratios (mean ± S.D.) of formulated feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and of the reference feed fresh mussel (FrM) fed to juvenile lobsters *Jasus edwardsii* over a 10 week growth trial.

	CO	FOL	FO	TO	SQM	MM	FrM[6]	F	P
	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.		
sum SFA[1]	18.5 ± 0.2 ^{a}	24.7 ± 0.3 ^{b}	31.1 ± 0.3 ^{c}	30.9 ± 1.6 ^{cd}	36.3 ± 3.0 ^{e}	35.1 ± 1.0 ^{de}	29.6 ± 0.8 ^{cd}	43.5	**<0.001**
sum MUFA[2]	52.1 ± 1.4 ^{d}	26.0 ± 1.1 ^{b}	26.1 ± 1.6 ^{b}	25.2 ± 0.7 ^{b}	34.0 ± 0.9 ^{c}	26.7 ± 0.5 ^{b}	10.6 ± 1.2 ^{a}	289.2	**<0.001**
sum PUFA[3]	25.3 ± 1.7 ^{a}	47.3 ± 2.0 ^{c}	40.4 ± 1.3 ^{bc}	39.3 ± 2.0 ^{b}	25.7 ± 2.2 ^{a}	32.0 ± 1.6 ^{a}	47.8 ± 3.3 ^{c}	37.7	**<0.001**
n-3[4]	14.0 ± 1.4 ^{a}	19.6 ± 0.4 ^{a}	35.0 ± 1.6 ^{c}	33.2 ± 2.2 ^{c}	18.7 ± 1.9 ^{a}	26.1 ± 1.2 ^{b}	41.5 ± 3.2 ^{d}	54.1	**<0.001**
n-6[5]	10.7 ± 3.1 ^{b}	26.5 ± 1.0 ^{c}	4.1 ± 0.4 ^{a}	5.5 ± 0.1 ^{a}	6.0 ± 0.3 ^{a}	4.3 ± 0.2 ^{a}	5.0 ± 1.0 ^{a}	84.4	**<0.001**
n-3/n-6	1.4 ± 0.5 ^{a}	0.7 ± 0.0 ^{a}	8.5 ± 0.4 ^{c}	6.1 ± 0.5 ^{bc}	3.1 ± 0.2 ^{ab}	6.1 ± 0.0 ^{bc}	8.5 ± 1.8 ^{c}	25.7	**<0.001**
EPA/ARA	9.2 ± 0.1 ^{a}	11.2 ± 0.0 ^{a}	13.2 ± 0.1 ^{a}	5.3 ± 0.1 ^{a}	12.6 ± 0.1 ^{a}	13.4 ± 0.2 ^{a}	95.2 ± 32.8 ^{b}	10.6	**0.002**
DHA/EPA	1.2 ± 0.0 ^{c}	1.1 ± 0.0 ^{c}	0.9 ± 0.1 ^{b}	2.3 ± 0.1 ^{e}	0.8 ± 0.0 ^{ab}	0.7 ± 0.0 ^{a}	1.5 ± 0.1 ^{d}	134.4	**<0.001**

Means that are not significantly different have the same superscript. Bold case indicates a significant one-way ANOVA result.

[1] Total saturated fatty acids as a percentage of the total fatty acids, includes: 14:0, i15:0, a15:0, i16:0, 16:0, 16:0 fatty aldehyde (FADE), i17:0, 17:0, 18:0, 18:0FADE, 20:0, 22:0.

[2] Total monounsaturated fatty acids as a percentage of the total fatty acids, includes: 16:1n-9c, 16:1n-7c, 16:1n-5c, 17:1, 17:1n-8, 18:1n-9c, 18:1n-7c, 18:1n-7t, 20:1n-9c, 20:1n-7c, 22:1n-11, 22:1n-9, 22:1n-7.

[3] Total polyunsaturated fatty acids as a percentage of the total fatty acids, includes: C16 PUFA, 18:3n-6, 18:4n-3, 18:2n-6, 20:4n-6, 20:5n-3, 20:3n-3, 20:2 non-methylene interrupted (NMI), 20:4n-3, 20:2n-6, 21:5n-6, 22:6n-3, 22:4n-6, 22:5n-3, 22:2NMI.

[4] Total n-3 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:4n-3, 20:5n-3, 20:4n-3, 21:5n-3, 22:6n-3, 22:5n-3.

[5] Total n-6 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:3n-6, 18:2n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6.

[6] Fresh mussel fatty acid composition from Murphy et al., 2002.

Table 6.4. Quantitative summary of fatty acid classes and essential fatty acid ratios (mg FA.g pellet DM^{-1}) (mean ± S.D., df=6,7) of formulated feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and of the reference feed fresh mussel (FrM).

	CO		FOL		FO		TO		SQM		MM		FrM[6]		F	P
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.		
sum SFA[1]	21.8	0.2 ^{a}	32.1	0.4 ^{b}	38.3	0.5 ^{b}	37.9	1.9 ^{b}	59.7	5.1 ^{c}	55.5	2.1 ^{c}	19.8	0.4 ^{a}	119.6	**<0.001**
sum MUFA[2]	61.4	1.7 ^{e}	34.2	1.3 ^{b}	32.4	1.5 ^{b}	31.3	0.9 ^{b}	56.6	1.6 ^{d}	42.2	0.8 ^{c}	8.6	1.3 ^{a}	422.3	**<0.001**
sum PUFA[3]	29.7	2.0 ^{a}	61.5	2.6 ^{d}	49.7	1.6 ^{bc}	48.1	2.5 ^{bc}	42.2	3.6 ^{b}	50.8	2.4 ^{c}	33.0	2.1 ^{a}	45.2	**<0.001**
n-3[4]	16.5	1.6 ^{a}	25.5	0.5 ^{b}	43.0	1.9 ^{c}	40.7	2.6 ^{c}	30.7	3.1 ^{b}	40.5	1.8 ^{c}	28.5	2.2 ^{b}	42.4	**<0.001**
n-6[5]	12.6	3.6 ^{c}	34.4	1.3 ^{d}	5.1	0.4 ^{ab}	6.7	0.1 ^{ab}	9.9	0.5 ^{bc}	6.6	0.3 ^{ab}	3.3	0.7 ^{a}	116.2	**<0.001**
n-3/n-6	1.4	0.5 ^{a}	0.7	0.0 ^{a}	8.5	0.4 ^{c}	6.1	0.5 ^{bc}	3.1	0.2 ^{ab}	6.1	0.0 ^{bc}	8.8	1.9 ^{c}	23.1	**<0.001**
EPA/ARA	9.2	0.1 ^{c}	11.2	0.0 ^{d}	13.2	0.1 ^{e}	5.3	0.1 ^{a}	12.6	0.1 ^{e}	13.4	0.2 ^{e}	8.5	0.5 ^{b}	293.9	**<0.001**
DHA/EPA	11.4	0.4 ^{ab}	12.3	0.1 ^{b}	11.8	0.9 ^{b}	12.2	0.5 ^{b}	10.4	0.5 ^{ab}	9.1	0.5 ^{a}	12.3	1.1 ^{b}	5.6	**0.015**

Means that are not significantly different have the same superscript. Bold case indicates a significant one-way ANOVA result.

[1] Total saturated fatty acids as a percentage of the total fatty acids, includes: 14:0, i15:0, a15:0, i16:0, 16:0, i17:0, 17:0, 18:0, 20:0, 22:0.

[2] Total monounsaturated fatty acids as a percentage of the total fatty acids, includes: 16:1n-9c, 16:1n-7c, 16:1n-5c, 17:1, 17:1n-8, 18:1n-9c (coeluted with 18:3n-3), 18:1n-7c, 18:1n-7t, 20:1n-9c, 20:1n-7c, 22:1n-11, 22:1n-9, 22:1n-7.

[3] Total polyunsaturated fatty acids as a percentage of the total fatty acids, includes: C16 PUFA, 18:3n-6, 18:4n-3, 18:2n-6, 20:4n-6, 20:5n-3, 20:3n-3, 20:2NMI, 20:4n-3, 20:2n-6, 21:5n-6, 22:6n-3, 22:4n-6, 22:5n-3, 22:2NMI.

[4] Total n-3 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:4n-3, 20:5n-3, 20:4n-3, 21:5n-3, 22:6n-3, 22:5n-3.

[5] Total n-6 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:3n-6, 18:2n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6.

[6] Fresh mussel fatty acid composition from Murphy et al., 2002.

Table 6.5. Percentage sterol and vitamin A composition (percentage total lipid) (mean ± S.D.) of formulated feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and of the reference feed fresh mussel (FrM) fed to juvenile lobsters *Jasus edwardsii* over a 10 week growth trial.

Sterol[1]	CO	FOL	FO	TO	SQM	MM	FrM
	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.
1	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.19 ± 0.00	0.39 ± 0.56
7	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.02	0.00 ± 0.00	0.01 ± 0.02	0.14 ± 0.00	0.35 ± 0.00
9	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00	0.06 ± 0.00	0.47 ± 0.00	2.28 ± 0.06
12[2]	3.35 ± 0.08	3.57 ± 0.24	4.16 ± 0.29	3.98 ± 0.22	3.08 ± 0.01	2.81 ± 0.00	7.77 ± 0.06
14	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.02	0.41 ± 0.07	0.02 ± 0.02	0.08 ± 0.11
30	0.06 ± 0.00	0.00 ± 0.00	0.04 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.61 ± 0.03	1.95 ± 0.13
33	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.19 ± 0.17	0.41 ± 0.58
41	0.23 ± 0.00	0.04 ± 0.06	0.16 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.09	0.29 ± 0.42
50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.29
vitamin A	0.28 ± 0.01	0.18 ± 0.02	0.17 ± 0.24	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.13	0.29 ± 0.40

[1] Scientific names of sterol species are given in Table 6.6.
[2] Cholesterol

Table 6.6. Sterol abbreviations (Table 6.5), trivial and scientific names (from Jones et al., 1994).

Abbreviation	Trivial name	Scientific name
1	24-norhydrocholesterol	24-norcholesta-5,22E-dien-3β-ol
7	patinosterol	27-nor-24-methyl-5α-cholest-22E-en-3β-ol
9	*trans*-22-dehydrocholesterol	cholesta-5,22E-dien-3β-ol
12	cholesterol	cholest-5-en-3β-ol
14	cholestanol	5α-cholestan-3β-ol
30	24-methylenecholesterol	24-methylcholesta-5,24(28)-dien-3β-ol
33	campesterol/dihydrobrassicasterol	24-methylcholest-5-en-3β-ol
41	Stigmasterol/poriferasterol	24-ethylcholesta-5,22E-dien-3β-ol
50	24-ethylcholesterol	24-ethylcholest-5-en-3β-ol

Table 6.7. Percentage fatty acid composition (mean ± S.D.) of digestive glands from juvenile lobsters *Jasus edwardsii* fed feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and the reference feed fresh mussel (FrM) over a 10 week growth trial, and from the initial population of lobsters.

	CO	FOL	FO	TO	SQM	MM	FrM[2]	Initial
	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	Mean ± S.D.
14:0	3.5 ± 2.8	2.5 ± 0.1	3.1 ± 0.7	2.0 ± 0.2	1.4 ± 0.6	5.3 ± 0.1	1.1 ± 0.0	1.9 ± 0.1
i15:0	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	1.3 ± 1.5	0.2 ± 0.1	0.1 ± 0.0
15:0	0.4 ± 0.2	0.3 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.8 ± 0.0	1.0 ± 0.1	0.7 ± 0.2
C16PUFA	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	1.7 ± 2.1	0.2 ± 0.2	0.2 ± 0.0
16:1n-9c	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.1 ± 0.2	0.1 ± 0.0	0.1 ± 0.1
16:1n-7c	4.9 ± 3.1	3.9 ± 0.1	7.4 ± 0.2	5.2 ± 0.3	6.2 ± 0.3	8.6 ± 0.5	7.1 ± 1.9	4.0 ± 2.7
16:0	14.8 ± 6.1	12.7 ± 0.6	14.8 ± 1.5	14.6 ± 0.4	13.1 ± 0.5	19.2 ± 0.8	16.6 ± 0.5	18.4 ± 0.6
16:0 Fade	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	0.2 ± 0.1	0.4 ± 0.3	0.2 ± 0.0
i17:0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	1.6 ± 0.1	0.7 ± 0.3
a17:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.8 ± 0.0	0.4 ± 0.2
17:1n-8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.4	0.0 ± 0.0
17:0	0.6 ± 0.2	0.6 ± 0.1	1.2 ± 0.1	0.9 ± 0.2	0.6 ± 0.0	0.8 ± 1.0	1.3 ± 0.2	0.9 ± 0.1
18:4n-3	0.7 ± 0.6	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.0	0.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.1	1.2 ± 0.0
18:2n-6	4.7 ± 4.6	22.4 ± 0.8	1.3 ± 0.1	1.5 ± 0.1	2.5 ± 0.0	1.7 ± 0.1	1.0 ± 0.0	8.8 ± 0.6
18:1n-9c[4]	26.4 ± 18.1	18.3 ± 0.2	16.5 ± 0.2	16.9 ± 0.5	23.4 ± 0.8	11.1 ± 0.1	11.0 ± 0.0	13.9 ± 0.8
18:1n-7c	4.8 ± 1.0	3.3 ± 0.1	5.3 ± 0.4	4.3 ± 0.4	5.5 ± 0.2	5.0 ± 0.4	7.1 ± 5.4	3.9 ± 0.1
18:1(n-7)t	0.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.3	0.3 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	1.9 ± 2.3	0.2 ± 0.2
18:0	6.3 ± 1.9	5.4 ± 0.3	8.6 ± 0.0	7.4 ± 0.0	6.9 ± 0.8	8.8 ± 0.1	7.5 ± 0.6	6.6 ± 0.6
18:0 FADE	0.3 ± 0.2	0.2 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.9 ± 0.3	0.5 ± 0.1	1.3 ± 0.0	0.7 ± 0.2
20:4n-6	1.0 ± 0.0	1.0 ± 0.2	1.5 ± 0.1	2.6 ± 0.3	1.9 ± 0.7	1.1 ± 0.1	2.6 ± 0.3	2.3 ± 0.3
20:5n-3	7.9 ± 2.3	7.7 ± 0.1	13.1 ± 1.3	12.0 ± 0.3	12.1 ± 2.4	9.5 ± 0.9	4.9 ± 5.8	9.4 ± 0.6
20:3n-6	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.7 ± 0.0	1.1 ± 1.2	0.6 ± 0.2
20:4n-3	0.8 ± 0.5	0.7 ± 0.2	1.3 ± 0.5	0.7 ± 0.2	5.4 ± 7.0	1.1 ± 0.2	1.8 ± 0.5	0.6 ± 0.3
20:2n-6	1.5 ± 0.8	1.7 ± 1.9	1.1 ± 0.1	1.5 ± 0.0	2.1 ± 0.1	0.9 ± 0.1	0.6 ± 0.0	0.0 ± 0.0
20:1(n-9)c	3.5 ± 0.2	2.5 ± 1.3	2.4 ± 0.2	2.9 ± 0.4	2.0 ± 0.1	3.5 ± 0.1	5.2 ± 0.3	4.6 ± 0.6
20:1(n-7)c	0.7 ± 0.3	0.6 ± 0.3	0.7 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	1.3 ± 0.0	2.2 ± 0.0	0.9 ± 0.3
20:0	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.1	0.6 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
21:5n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	0.0 ± 0.0

22:5n-6	0.4 ± 0.4	0.2 ± 0.0	0.2 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.0
22:6n-3	10.0 ± 1.5	9.7 ± 0.3	12.2 ± 1.3	16.7 ± 0.1	9.5 ± 1.9	7.7 ± 0.1	9.6 ± 0.5	12.0 ± 1.0
22:5n-3	1.6 ± 0.6	1.4 ± 0.1	1.8 ± 0.3	0.7 ± 0.9	0.9 ± 0.1	1.6 ± 0.0	1.1 ± 0.1	1.5 ± 0.0
22:1(n-11)	0.6 ± 0.4	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.4 ± 0.2	0.6 ± 0.1	0.3 ± 0.0	0.8 ± 0.0
22:1(n-9)	1.6 ± 1.4	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.3	0.1 ± 0.0	0.3 ± 0.0
22:1(n-7)	0.1 ± 0.2	0.2 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
22:2NMI	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.9 ± 0.5	0.0 ± 0.0
22:0	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Others[1]	2.4 ± 0.8	1.9 ± 0.1	3.1 ± 0.5	3.5 ± 0.1	2.1 ± 0.0	4.1 ± 0.1	5.2 ± 0.0[3]	4.4 ± 0.7

[1]Others includes fatty acids with less than 0.2%; i15:0, a15:0, i16:0, 16:1n-5c, 16:1n-7t, 17:1, 17:1n-6, i18:0, 18:1n-5c, i19:0, 19:1, 20:1n-11c, C21 PUFA C22 PUFA, 20:2NMI, 22:4n-6, 24:1, 24:0

[2] Fresh mussel fatty acid composition from Murphy et al., 2002.

[3]Others calculated from mean site data (n=3) in (Murphy et al., 2002), includes 17:2, C_{22} PUFA, Dimethylacetal (DMA) of 16:0 and 18:0 and 18:0 fatty aldehyde, 28:8n-3, C_{23}PUFA, 20:1n-11c, 20:2 NMI, 19:1, 18:5n-3, i18:0, 16:1n-7t, 4,8,12 trimethyl tetradecanoic acid (TMTD).

[4]Includes 18:3n-3

Table 6.8. Percentage fatty acid classes and essential fatty acid ratios (mean ± S.D.) of digestive glands from juvenile lobsters *Jasus edwardsii* fed feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and the reference feed fresh mussel (FrM) over a 10 week growth trial, and from the initial population of lobsters.

	CO	FOL	FO	TO	SQM	MM	FrM	Initial	F	P
	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.	mean ± S.D.		
sum SFA[1]	27.1 ± 12.1	22.7 ± 0.9	29.4 ± 2.7	26.9 ± 0.1	23.1 ± 0.7	38.3 ± 0.1	31.1 ± 1.1	31.0 ± 2.0	2.5	0.107
sum MUFA[2]	43.4 ± 12.3	30.3 ± 1.9	34.8 ± 0.4	32.7 ± 1.8	39.1 ± 0.3	32.2 ± 0.4	36.0 ± 8.7	29.5 ± 2.7	1.5	0.302
sum PUFA[3]	28.8 ± 0.8^{ab}	46.1 ± 0.9^{c}	33.8 ± 3.3^{ab}	38.0 ± 1.5^{bc}	35.3 ± 1.6^{ab}	27.8 ± 1.0^{ab}	25.5 ± 6.6^{a}	37.4 ± 0.1^{bc}	11.5	**0.001**
n-3[4]	20.9 ± 5.6	20.2 ± 0.5	29.2 ± 3.2	30.7 ± 1.1	28.3 ± 2.5	21.3 ± 1.0	18.8 ± 6.9	24.6 ± 0.2	3.5	0.051
n-6[5]	7.7 ± 4.9^{ab}	25.6 ± 1.4^{c}	4.5 ± 0.1^{a}	6.9 ± 0.4^{ab}	7.0 ± 0.9^{ab}	4.7 ± 0.1^{a}	6.2 ± 0.5^{ab}	12.6 ± 0.1^{b}	28.8	**<0.001**
n-3/n-6	3.7 ± 3.1^{ab}	0.8 ± 0.1^{a}	6.5 ± 0.6^{b}	4.4 ± 0.1^{ab}	4.1 ± 0.9^{ab}	4.5 ± 0.1^{ab}	3.1 ± 1.4^{ab}	1.9 ± 0.0^{ab}	3.8	**0.039**
EPA/ARA	8.2 ± 2.7^{b}	8.2 ± 1.2^{b}	8.7 ± 0.2^{b}	4.7 ± 0.4^{ab}	6.4 ± 1.0^{ab}	8.9 ± 0.1^{b}	1.8 ± 2.0^{a}	4.1 ± 0.8^{ab}	7.4	**0.006**
DHA/EPA	1.3 ± 0.2	1.3 ± 0.1	0.9 ± 0.0	1.4 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	6.3 ± 7.3	1.3 ± 0.2	1.0	0.487

Means that are not significantly different have the same superscript. Bold case indicates a significant one-way ANOVA result.

[1] Total saturated fatty acids as a percentage of the total fatty acids, includes: 14:0, i15:0, a15:0, i16:0, 16:0, i17:0, 17:0, 18:0, 20:0, 22:0.

[2] Total monounsaturated fatty acids as a percentage of the total fatty acids, includes: 16:1n-9c, 16:1n-7c, 16:1n-5c, 17:1, 17:1n-8, 18:1n-9c, 18:1n-7c, 18:1n-7t, 20:1n-9c, 20:1n-7c, 22:1n-11, 22:1n-9, 22:1n-7.

[3] Total polyunsaturated fatty acids as a percentage of the total fatty acids, includes: C16 PUFA, 18:3n-6, 18:4n-3, 18:2n-6, 20:4n-6, 20:5n-3, 20:3n-3, 20:2NMI, 20:4n-3, 20:2n-6, 21:5n-6, 22:6n-3, 22:4n-6, 22:5n-3, 22:2NMI.

[4] Total n-3 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:4n-3, 20:5n-3, 20:4n-3, 21:5n-3, 22:6n-3, 22:5n-3.

[5] Total n-6 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:3n-6, 18:2n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6.

Table 6.9. Quantitative fatty acid (FA) composition (mg FA.g wet tissue^{-1}) (mean ± S.D.) of digestive gland fatty acids of juvenile lobsters *Jasus edwardsii* feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and of the reference feed fresh mussel (FrM) over a 10 week growth trial.

	CO		FOL		FO		TO		SQM		MM		FrM[2]		Initial	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
14:0	2.9	± 2.4	2.3	± 0.1	1.1	± 0.2	0.7	± 0.1	0.3	± 0.1	5.2	± 0.1	1.3	± 0.0	1.9	± 0.1
i15:0	0.2	± 0.1	0.1	± 0.0	0.1	± 0.0	0.0	± 0.0	0.0	± 0.0	1.3	± 1.5	0.2	± 0.2	0.1	± 0.0
15:0	0.3	± 0.2	0.3	± 0.0	0.1	± 0.0	0.2	± 0.0	0.1	± 0.0	0.8	± 0.0	1.3	± 0.1	0.7	± 0.2
C16PUFA	0.1	± 0.1	0.2	± 0.0	0.1	± 0.0	0.0	± 0.0	0.0	± 0.0	1.7	± 2.1	0.2	± 0.3	0.2	± 0.0
16:1n-9c	0.3	± 0.1	0.2	± 0.0	0.1	± 0.0	0.1	± 0.0	0.1	± 0.0	0.1	± 0.2	0.1	± 0.1	0.1	± 0.1
16:1n-7c	4.0	± 2.6	3.5	± 0.1	2.6	± 0.1	1.9	± 0.1	1.4	± 0.1	8.5	± 0.5	8.8	± 2.3	4.0	± 2.7
16:0	12.3	± 5.1	11.5	± 0.5	5.2	± 0.5	5.3	± 0.1	2.9	± 0.1	19.0	± 0.8	20.4	± 0.6	18.4	± 0.6
16:0 Fade	0.2	± 0.1	0.2	± 0.1	0.2	± 0.0	0.2	± 0.0	0.2	± 0.0	0.2	± 0.1	0.5	± 0.4	0.2	± 0.0
i17:0	0.2	± 0.1	0.1	± 0.0	0.1	± 0.0	0.1	± 0.0	0.0	± 0.0	0.6	± 0.0	1.9	± 0.1	0.7	± 0.3
a17:0	0.1	± 0.0	0.1	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.2	± 0.0	1.0	± 0.0	0.4	± 0.2
17:1n-8	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.1	± 0.2	0.0	± 0.0	0.0	± 0.0	0.3	± 0.5	0.0	± 0.0
17:0	0.5	± 0.2	0.5	± 0.1	0.4	± 0.0	0.3	± 0.1	0.1	± 0.0	0.8	± 1.0	1.6	± 0.2	0.9	± 0.1
18:3n-6	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.1	± 0.0	0.4	± 0.0	0.2	± 0.1
18:4n-3	0.6	± 0.5	0.6	± 0.1	0.3	± 0.0	0.2	± 0.0	0.1	± 0.0	1.4	± 0.0	1.7	± 0.1	1.2	± 0.0
18:2n-6	3.9	± 3.8	20.2	± 0.7	0.5	± 0.0	0.6	± 0.0	0.6	± 0.0	1.6	± 0.1	1.2	± 0.0	8.8	± 0.6
18:1n-9c[4]	21.9	± 15.0	16.5	± 0.2	5.8	± 0.1	6.2	± 0.2	5.2	± 0.2	11.0	± 0.1	13.6	± 0.1	13.9	± 0.8
18:1n-7c	4.0	± 0.8	3.0	± 0.1	1.8	± 0.1	1.6	± 0.1	1.2	± 0.0	4.9	± 0.4	8.8	± 6.7	3.9	± 0.1
18:1(n-7)t	0.1	± 0.1	0.1	± 0.2	0.1	± 0.1	0.1	± 0.0	0.0	± 0.0	0.0	± 0.0	2.3	± 2.8	0.2	± 0.2
18:0	5.2	± 1.5	4.8	± 0.3	3.0	± 0.0	2.7	± 0.0	1.5	± 0.2	8.7	± 0.1	9.3	± 0.7	6.6	± 0.6
18:0 FAde	0.2	± 0.1	0.2	± 0.1	0.2	± 0.0	0.2	± 0.1	0.2	± 0.1	0.5	± 0.1	1.7	± 0.1	0.7	± 0.2
20:4n-6	0.8	± 0.0	0.9	± 0.1	0.5	± 0.0	0.9	± 0.1	0.4	± 0.1	1.1	± 0.1	3.2	± 0.4	2.3	± 0.3
20:5n-3	6.5	± 1.9	7.0	± 0.1	4.6	± 0.5	4.4	± 0.1	2.7	± 0.5	9.4	± 0.9	6.0	± 7.1	9.4	± 0.6
20:3n-6	0.1	± 0.1	0.1	± 0.0	0.1	± 0.0	0.1	± 0.0	0.0	± 0.0	0.7	± 0.0	1.4	± 1.5	0.6	± 0.2
20:4n-3	0.6	± 0.4	0.6	± 0.2	0.4	± 0.2	0.3	± 0.1	1.2	± 1.6	1.1	± 0.2	2.2	± 0.6	0.6	± 0.3
20:2n-6	1.2	± 0.7	1.6	± 1.7	0.4	± 0.0	0.5	± 0.0	0.5	± 0.0	0.9	± 0.1	0.8	± 0.0	0.0	± 0.0
20:1(n-9)c	2.9	± 0.2	2.3	± 1.1	0.8	± 0.1	1.1	± 0.1	0.4	± 0.0	3.5	± 0.1	6.4	± 0.4	4.6	± 0.6
20:1(n-7)c	0.6	± 0.2	0.5	± 0.3	0.3	± 0.0	0.2	± 0.0	0.1	± 0.0	1.3	± 0.0	2.7	± 0.1	0.9	± 0.3
20:0	0.4	± 0.1	0.3	± 0.0	0.1	± 0.0	0.2	± 0.0	0.1	± 0.0	0.6	± 0.0	0.3	± 0.0	0.3	± 0.0

21:5n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	0.0 ± 0.0
22:5n-6	0.4 ± 0.3	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.4 ± 0.0
22:6n-3	8.3 ± 1.3	8.7 ± 0.3	4.3 ± 0.4	6.1 ± 0.0	2.1 ± 0.4	7.6 ± 0.1	11.8 ± 0.6	12.0 ± 1.0
22:5n-3	1.3 ± 0.5	1.3 ± 0.1	0.6 ± 0.1	0.3 ± 0.3	0.2 ± 0.0	1.6 ± 0.0	1.4 ± 0.1	1.5 ± 0.0
22:1(n-11)	0.5 ± 0.3	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	0.4 ± 0.0	0.8 ± 0.0
22:1(n-9)	1.3 ± 1.1	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.5 ± 0.3	0.1 ± 0.0	0.3 ± 0.0
22:1(n-7)	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
22:2NMI	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.8 ± 0.6	0.0 ± 0.0
22:0	0.1 ± 0.1	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Others[1]	1.2 ± 0.7	3.4 ± 0.0	0.6 ± 0.2	0.1 ± 0.1	0.2 ± 0.0	1.4 ± 0.1	0.2 ± 0.1[3]	0.1 ± 0.7

[1]Others includes fatty acids with less than 0.2%; i15:0, a15:0, i16:0, 16:1n-5c, 16:1n-7t, 17:1, 17:1n-6, i18:0, 18:1n-5c, i19:0, 19:1, 20:1n-11c, C21 PUFA C22 PUFA, 20:2NMI, 22:4n-6, 24:1, 24:0

[2] Fresh mussel fatty acid composition from Murphy et al., 2002.

[3]Others calculated from mean site data (n=3) in (Murphy et al., 2002), includes 17:2, C_{22} PUFA, Dimethylacetal (DMA) of 16:0 and 18:0 and 18:0 fatty aldehyde, 28:8n-3, C_{23}PUFA, 20:1n-11c, 20:2 NMI, 19:1, 18:5n-3, i18:0, 16:1n-7t, 4,8,12 trimethyl tetradecanoic acid (TMTD).

[4]Includes 18:3n-3

Table 6.10. Quantitative summary of fatty acid classes and essential fatty acid ratios (mg FA.g pellet DM^{-1}) (mean ± S.D., df=7,8) of digestive glands from juvenile lobsters *Jasus edwardsii* fed feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and of the reference feed fresh mussel (FrM) over a 10 week growth trial.

	CO		FOL		FO		TO		SQM		MM		FrM		Initial		F	P
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.		
sum SFA[1]	22.7 ±	9.9 ^{bc}	20.7 ±	0.8 ^{bc}	10.5 ±	0.9 ^{ab}	10.1 ±	0.0 ^{ab}	5.4 ±	0.2 ^{a}	38.4 ±	0.2 ^{d}	40.0 ±	1.4 ^{d}	31.7 ±	2.2 ^{cd}	26.7	**<0.001**
sum MUFA[2]	36.0 ±	10.2 ^{d}	27.3 ±	1.7 ^{abc}	12.2 ±	0.1 ^{ab}	12.1 ±	0.7 ^{ab}	8.8 ±	0.1 ^{a}	31.8 ±	0.4 ^{cd}	44.9 ±	10.7 ^{d}	29.5 ±	2.7 ^{cd}	11.8	**0.001**
sum PUFA[3]	23.9 ±	0.7 ^{b}	41.6 ±	0.8 ^{d}	11.8 ±	1.1 ^{a}	13.9 ±	0.5 ^{a}	7.9 ±	0.4 ^{a}	27.5 ±	1.0 ^{bc}	36.3 ±	8.8 ^{cd}	37.4 ±	0.1 ^{cd}	32.7	**<0.001**
n-3[4]	17.4 ±	4.6 ^{abc}	18.3 ±	0.5 ^{abc}	10.2 ±	1.1 ^{ab}	11.3 ±	0.4 ^{abc}	6.3 ±	0.6 ^{a}	21.1 ±	1.0 ^{bc}	23.6 ±	8.5 ^{c}	24.6 ±	0.2 ^{c}	7.3	**0.006**
n-6[5]	6.4 ±	4.1 ^{ab}	23.1 ±	1.3 ^{d}	1.6 ±	0.0 ^{a}	2.5 ±	0.2 ^{ab}	1.6 ±	0.2 ^{a}	4.7 ±	0.1 ^{ab}	7.7 ±	0.5 ^{b}	12.6 ±	0.1 ^{c}	46.3	**<0.001**
n-3/n-6	3.7 ±	3.1 ^{ab}	0.8 ±	0.1 ^{a}	6.5 ±	0.6 ^{b}	4.5 ±	0.1 ^{ab}	4.1 ±	0.9 ^{ab}	4.5 ±	0.1 ^{ab}	3.1 ±	1.3 ^{ab}	1.9 ±	0.0 ^{ab}	3.9	**0.038**
EPA/ARA	8.2 ±	2.7 ^{b}	8.2 ±	1.2 ^{b}	8.7 ±	0.2 ^{b}	4.7 ±	0.4 ^{ab}	6.4 ±	1.0 ^{ab}	8.9 ±	0.1 ^{b}	1.8 ±	2.0 ^{a}	4.1 ±	0.8 ^{ab}	7.4	**0.006**
DHA/EPA	10.4 ±	2.0 ^{c}	10.3 ±	2.0 ^{c}	8.2 ±	0.2 ^{bc}	6.5 ±	0.7 ^{abc}	5.1 ±	0.7 ^{ab}	7.2 ±	0.9 ^{abc}	3.7 ±	0.3 ^{a}	5.1 ±	0.2 ^{ab}	9.7	**0.002**

Means that are not significantly different have the same superscript. Bold case indicates a significant one-way ANOVA result.

[1] Total saturated fatty acids as a percentage of the total fatty acids, includes: 14:0, i15:0, a15:0, i16:0, 16:0, i17:0, 17:0, 18:0, 20:0, 22:0.

[2] Total monounsaturated fatty acids as a percentage of the total fatty acids, includes: 16:1n-9c, 16:1n-7c, 16:1n-5c, 17:1, 17:1n-8, 18:1n-9c, 18:1n-7c, 18:1n-7t, 20:1n-9c, 20:1n-7c, 22:1n-11, 22:1n-9, 22:1n-7.

[3] Total polyunsaturated fatty acids as a percentage of the total fatty acids, includes: C16 PUFA, 18:3n-6, 18:4n-3, 18:2n-6, 20:4n-6, 20:5n-3, 20:3n-3, 20:2NMI, 20:4n-3, 20:2n-6, 21:5n-6, 22:6n-3, 22:4n-6, 22:5n-3, 22:2NMI.

[4] Total n-3 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:4n-3, 20:5n-3, 20:4n-3, 21:5n-3, 22:6n-3, 22:5n-3.

[5] Total n-6 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:3n-6, 18:2n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6.

Table 6.11. Muscle tissue fatty acid percentage composition (%) (mean ± S.D.) of muscle tissue from juvenile lobsters *Jasus edwardsii* fed feeds containing squid meal (SQM) and mussel meal (MM) over a 10 week growth trial, and from the initial population of lobsters.

	SQM		MM		Initial	
	mean	± S.D.	mean	± S.D.	mean	± S.D.
14:0	0.9	± 0.0	1.6	± 0.0	0.6	± 0.1
15:0	0.4	± 0.0	0.5	± 0.0	0.7	± 0.1
16:1n-9c	0.2	± 0.0	0.1	± 0.0	0.0	± 0.1
16:1n-7c	5.2	± 0.2	7.4	± 0.0	4.6	± 0.3
16:0	15.7	± 0.2	16.8	± 0.3	16.4	± 0.6
16:0 Fade	0.6	± 0.1	0.4	± 0.0	0.5	± 0.0
i17:0	0.2	± 0.0	0.3	± 0.0	0.5	± 0.0
a17:0	0.1	± 0.0	0.1	± 0.0	0.3	± 0.0
17:0	0.9	± 0.2	1.1	± 0.1	1.3	± 0.1
18:4n-3	0.8	± 0.1	1.1	± 0.4	1.0	± 0.0
18:2n-6	2.3	± 0.1	1.5	± 0.2	4.0	± 0.0
18:1n-9c[2]	18.1	± 0.7	13.3	± 0.2	13.1	± 0.1
18:1n-7c	4.3	± 0.1	3.7	± 0.2	3.0	± 0.0
18:1(n-7)t	0.4	± 0.0	0.3	± 0.0	0.2	± 0.0
18:1(n-5)c	0.2	± 0.0	0.2	± 0.0	0.2	± 0.0
18:0	7.5	± 0.1	10.0	± 0.2	7.8	± 0.1
18:0 FAde	0.8	± 0.0	1.1	± 0.2	1.4	± 0.0
19:1	0.2	± 0.0	0.1	± 0.0	0.2	± 0.0
20:4n-6	3.2	± 0.2	2.3	± 0.1	5.0	± 0.1
20:5n-3	16.0	± 0.5	16.9	± 0.2	14.4	± 0.3
20:3n-6	0.1	± 0.0	0.3	± 0.0	0.7	± 0.0
20:4n-3	0.3	± 0.0	0.5	± 0.0	0.0	± 0.0
20:2n-6	1.3	± 0.1	0.7	± 0.0	0.8	± 0.0
20:1(n-9)c	2.1	± 0.1	2.1	± 0.1	2.5	± 0.0
20:1(n-7)c	0.3	± 0.0	0.6	± 0.0	0.6	± 0.0
20:0	0.9	± 0.0	0.7	± 0.1	0.7	± 0.0
22:5n-6	0.2	± 0.0	0.2	± 0.0	0.4	± 0.0
22:6n-3	13.4	± 0.4	11.6	± 0.0	14.5	± 0.8
22:4n-6	0.0	± 0.0	0.1	± 0.1	0.4	± 0.0
22:5n-3	0.5	± 0.0	1.3	± 0.1	1.0	± 0.0
22:1(n-11)	0.1	± 0.0	0.2	± 0.0	0.2	± 0.0
22:1(n-9)	0.2	± 0.0	0.1	± 0.0	0.1	± 0.0
22:1(n-7)	0.1	± 0.0	0.2	± 0.0	0.1	± 0.0
22:0	1.0	± 0.0	0.5	± 0.1	0.7	± 0.0
Others[1]	1.3	± 0.1	1.7	± 0.6	1.8	± 0.1

[1]Others includes fatty acids with less than 0.2%; i15:0, a15:0, i16:0, 16:1n-5c, 16:1n-7t, 17:1, 17:1n-6, i18:0, 18:1n-5c, i19:0, 19:1, 20:1n-11c, C21 PUFA C22 PUFA, 20:2NMI, 22:4n-6, 24:1, 24:0

[2]Includes 18:3n-3

Table 6.12. Percentage fatty acid classes and essential fatty acid ratios (mean ± S.D., df=2,3) of muscle tissue from juvenile lobsters *Jasus edwardsii* fed feeds containing squid meal (SQM) and mussel meal (MM) over a 10 week growth trial, and from the initial population of lobsters.

	SQM	MM	Initial	F	P
	mean ± S.D.	mean ± S.D.	mean ± S.D.		
sum SFA[1]	28.0 ± 0.0 [a]	32.3 ± 0.2 [b]	29.5 ± 0.9 [a]	32.1	**0.009**
sum MUFA[2]	31.5 ± 1.1 [c]	28.4 ± 0.2 [b]	25.1 ± 0.3 [a]	45.7	**0.006**
sum PUFA[3]	38.3 ± 1.1 [a]	36.6 ± 0.3 [a]	42.3 ± 1.3 [b]	16.18	**0.025**
n-3[4]	31.0 ± 0.7	31.4 ± 0.5	30.9 ± 1.2	0.174	0.848
n-6[5]	7.3 ± 0.3 [b]	5.2 ± 0.1 [a]	11.4 ± 0.1 [c]	473.8	**<0.001**
n-3/n-6	4.3 ± 0.1 [b]	6.0 ± 0.2 [c]	2.7 ± 0.1 [a]	239.0	**<0.001**
EPA/ARA	5.0 ± 0.1 [b]	7.5 ± 0.1 [c]	2.9 ± 0.0 [a]	1477.6	**<0.001**
DHA/EPA	0.8 ± 0.0 [b]	0.7 ± 0.0 [a]	1.0 ± 0.0 [c]	126.2	**0.001**

Means that are not significantly different have the same superscript. Bold case indicates a significant one-way ANOVA result.

[1] Total saturated fatty acids as a percentage of the total fatty acids, includes: 14:0, i15:0, a15:0, i16:0, 16:0, i17:0, 17:0, 18:0, 20:0, 22:0.

[2] Total monounsaturated fatty acids as a percentage of the total fatty acids, includes: 16:1n-9c, 16:1n-7c, 16:1n-5c, 17:1, 17:1n-8, 18:1n-9c, 18:1n-7c, 18:1n-7t, 20:1n-9c, 20:1n-7c, 22:1n-11, 22:1n-9, 22:1n-7.

[3] Total polyunsaturated fatty acids as a percentage of the total fatty acids, includes: C16 PUFA, 18:3n-6, 18:4n-3, 18:2n-6, 20:4n-6, 20:5n-3, 20:3n-3, 20:2NMI, 20:4n-3, 20:2n-6, 21:5n-6, 22:6n-3, 22:4n-6, 22:5n-3, 22:2NMI.

[4] Total n-3 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:4n-3, 20:5n-3, 20:4n-3, 21:5n-3, 22:6n-3, 22:5n-3.

[5] Total n-6 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:3n-6, 18:2n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6.

Table 6.13.Quantitative fatty acid (FA) composition (mg FA.g wet tissue^{-1}) (mean ± S.D.) of muscle tissue from juvenile lobsters *Jasus edwardsii* fed feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and of the reference feed fresh mussel (FrM) over a 10 week growth trial.

	SQM		MM		Initial	
	mean	S.D.	mean	S.D.	mean	S.D.
14:0	0.1	0.0	0.3	0.0	0.1	0.0
i15:0	0.0	0.0	0.0	0.0	0.0	0.0
15:0	0.0	0.0	0.1	0.0	0.1	0.0
C16PUFA	0.0	0.0	0.0	0.0	0.0	0.0
16:1n-9c	0.0	0.0	0.0	0.0	0.0	0.0
16:1n-7c	0.3	0.0	1.2	0.0	0.5	0.0
16:0	1.0	0.0	2.6	0.0	1.7	0.1
16:0 Fade	0.0	0.0	0.1	0.0	0.0	0.0
i17:0	0.0	0.0	0.0	0.0	0.1	0.0
a17:0	0.0	0.0	0.0	0.0	0.0	0.0
17:1n-8	0.0	0.0	0.0	0.0	0.0	0.0
17:0	0.1	0.0	0.2	0.0	0.1	0.0
18:3n-6	0.0	0.0	0.0	0.0	0.0	0.0
18:4n-3	0.1	0.0	0.2	0.1	0.1	0.0
18:2n-6	0.1	0.0	0.2	0.0	0.4	0.0
18:1n-9c[2]	1.2	0.0	2.1	0.0	1.4	0.0
18:1n-7c	0.3	0.0	0.6	0.0	0.3	0.0
18:1(n-7)t	0.0	0.0	0.0	0.0	0.0	0.0
18:0	0.5	0.0	1.5	0.0	0.8	0.0
18:0 FAde	0.1	0.0	0.2	0.0	0.1	0.0
20:4n-6	0.2	0.0	0.3	0.0	0.5	0.0
20:5n-3	1.0	0.0	2.6	0.0	1.5	0.0
20:3n-6	0.0	0.0	0.1	0.0	0.1	0.0
20:4n-3	0.0	0.0	0.1	0.0	0.0	0.0
20:2n-6	0.1	0.0	0.1	0.0	0.1	0.0
20:1(n-9)c	0.1	0.0	0.3	0.0	0.3	0.0
20:1(n-7)c	0.0	0.0	0.1	0.0	0.1	0.0
20:0	0.1	0.0	0.1	0.0	0.1	0.0
21:5n-3	0.0	0.0	0.0	0.0	0.0	0.0
22:5n-6	0.0	0.0	0.0	0.0	0.0	0.0
22:6n-3	0.9	0.0	1.8	0.0	1.5	0.1
22:5n-3	0.0	0.0	0.2	0.0	0.1	0.0
22:1(n-11)	0.0	0.0	0.0	0.0	0.0	0.0
22:1(n-9)	0.0	0.0	0.0	0.0	0.0	0.0
22:1(n-7)	0.0	0.0	0.0	0.0	0.0	0.0
22:2NMI	0.0	0.0	0.0	0.0	0.0	0.0
22:0	0.1	0.0	0.1	0.0	0.1	0.0
others	0.1	0.0	0.7	0.1	0.5	0.0

[1]Others includes fatty acids with less than 0.2%; i15:0, a15:0, i16:0, 16:1n-5c, 16:1n-7t, 17:1, 17:1n-6, i18:0, 18:1n-5c, i19:0, 19:1, 20:1n-11c, C21 PUFA C22 PUFA, 20:2NMI, 22:4n-6, 24:1, 24:0

[2]Includes 18:3n-3

Table 6.14. Quantitative summary of fatty acid classes and essential fatty acid ratios (mg FA.g pellet DM^{-1}) (mean ± S.D., df=2, 3) of muscle tissues from juvenile lobsters *Jasus edwardsii* fed feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and of the reference feed fresh mussel (FrM) over a 10 week growth trial.

	SQM		MM		IN		F	P
	mean	S.D.	mean	S.D.	mean	S.D.		
sum SFA[1]	1.8	± 0.0[a]	5.2	± 0.0[c]	3.2	± 0.1[b]	1822.0	**<0.001**
sum MUFA[2]	2.0	± 0.1[a]	4.4	± 0.0[c]	2.6	± 0.0[b]	1305.4	**<0.001**
sum PUFA[3]	2.5	± 0.1[a]	5.7	± 0.0[c]	4.4	± 0.1[b]	637.2	**<0.001**
n-3[4]	2.0	± 0.0[a]	4.9	± 0.1[c]	3.2	± 0.1[b]	528.6	**<0.001**
n-6[5]	0.5	± 0.0[a]	0.8	± 0.0[b]	1.2	± 0.0[c]	854.3	**<0.001**
n-3/n-6	4.3	± 0.1[b]	6.0	± 0.2[c]	2.7	± 0.1[a]	239.0	**<0.001**
EPA/ARA	5.0	± 0.1[b]	7.5	± 0.1[c]	2.9	± 0.0[a]	1477.6	**<0.001**
DHA/EPA	4.1	± 0.1[b]	5.1	± 0.1[c]	2.9	± 0.1[a]	177.6	**0.001**

Means that are not significantly different have the same superscript. Bold case indicates a significant one-way ANOVA result.

[1] Total saturated fatty acids as a percentage of the total fatty acids, includes: 14:0, i15:0, a15:0, i16:0, 16:0, i17:0, 17:0, 18:0, 20:0, 22:0.

[2] Total monounsaturated fatty acids as a percentage of the total fatty acids, includes: 16:1n-9c, 16:1n-7c, 16:1n-5c, 17:1, 17:1n-8, 18:1n-9c, 18:1n-7c, 18:1n-7t, 20:1n-9c, 20:1n-7c, 22:1n-11, 22:1n-9, 22:1n-7.

[3] Total polyunsaturated fatty acids as a percentage of the total fatty acids, includes: C16 PUFA, 18:3n-6, 18:4n-3, 18:2n-6, 20:4n-6, 20:5n-3, 20:3n-3, 20:2NMI, 20:4n-3, 20:2n-6, 21:5n-6, 22:6n-3, 22:4n-6, 22:5n-3, 22:2NMI.

[4] Total n-3 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:4n-3, 20:5n-3, 20:4n-3, 21:5n-3, 22:6n-3, 22:5n-3.

[5] Total n-6 fatty acids as a percentage of the total fatty acids from the total lipid extract, includes: 18:3n-6, 18:2n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6.

[6] Fresh mussel fatty acid composition from Murphy et al., 2002.

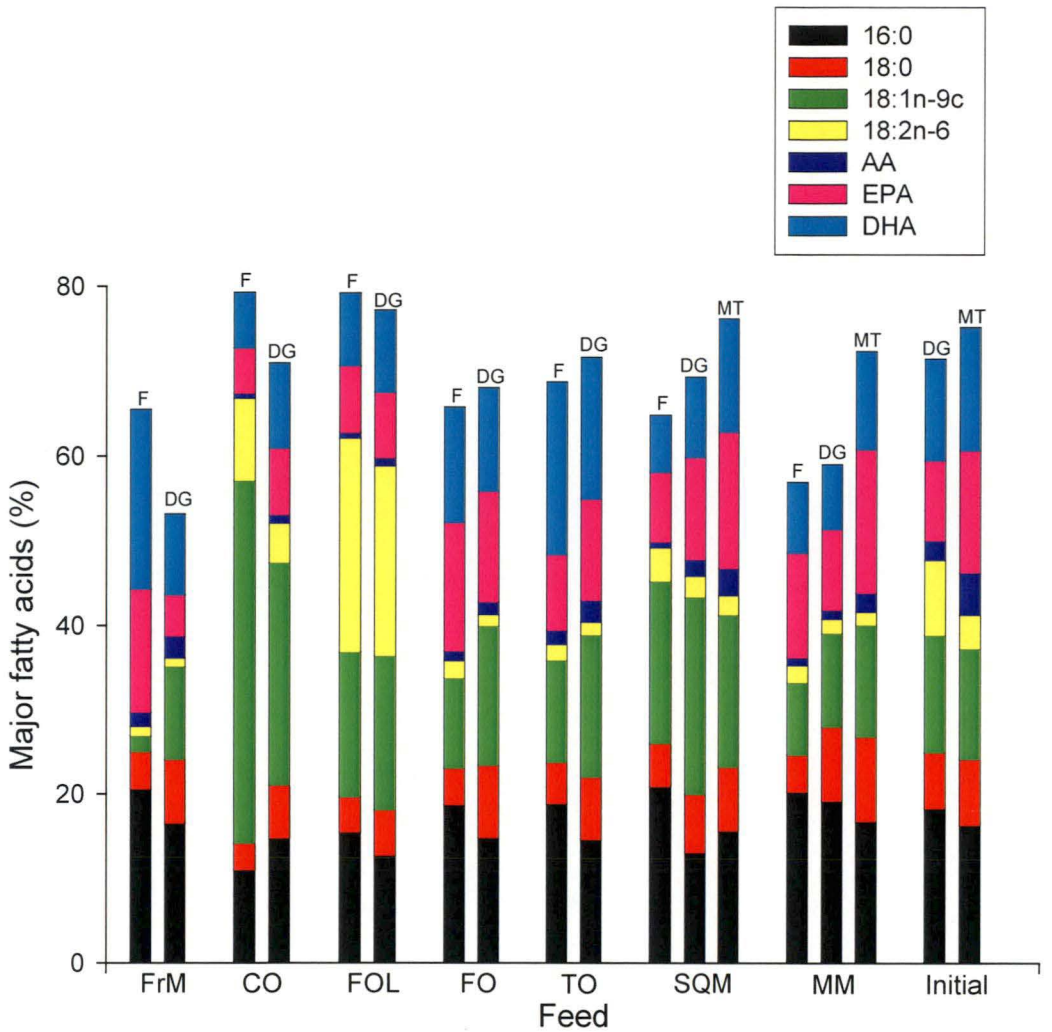

Figure 6.1. The major fatty acids as a percentage of total fatty acids (%) in the feed (F), digestive gland (DG) and muscle tissue (MT) in juvenile *J. edwardsii* after feeding for 10 weeks. Fresh mussel fed lobsters and initial compositions are included for reference. Lobsters (n=5) from within each formulated feed treatment (n=3) and fresh mussel feed (n=2) were pooled and analysed in duplicate for all samples. * Fresh mussel composition was calculated from the fatty acid profile from Murphy et al., (2002).

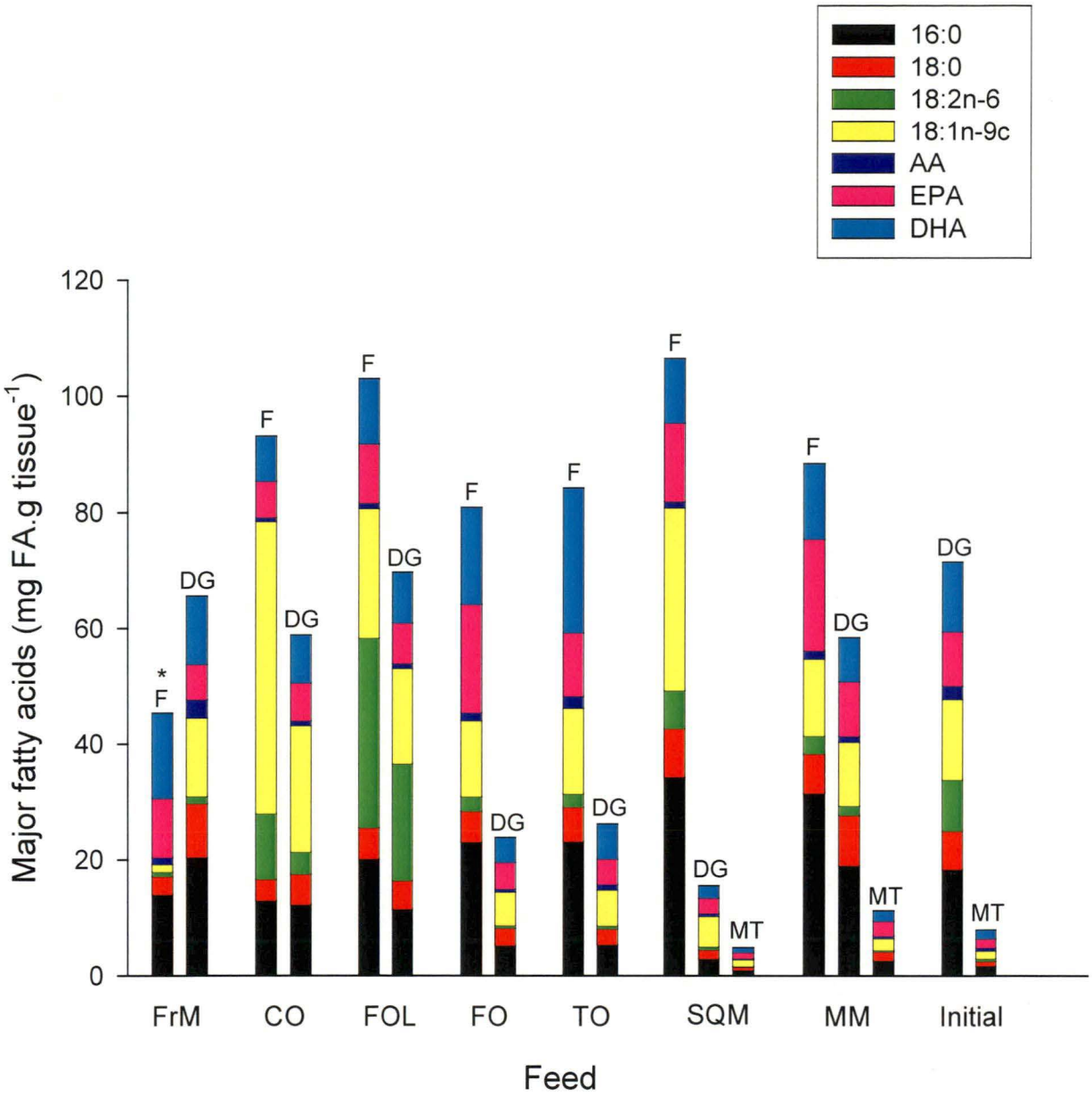

Figure 6.2. Quantitative summary of major fatty acid (FA) classes (mg FA.g wet tissue^{-1}) of feeds (F), digestive gland (DG) and muscle tissues (MT) from juvenile lobsters *Jasus edwardsii* fed feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and the reference feed fresh mussel (FrM) over a 10 week growth trial. Fresh mussel fed lobsters and initial compositions are included for reference. Lobsters (n=5) from within each formulated feed treatment (n=3) and fresh mussel feed (n=2) were pooled and analysed in duplicate for all samples. * Fresh mussel composition was calculated from the fatty acid profile from Murphy et al., (2002).

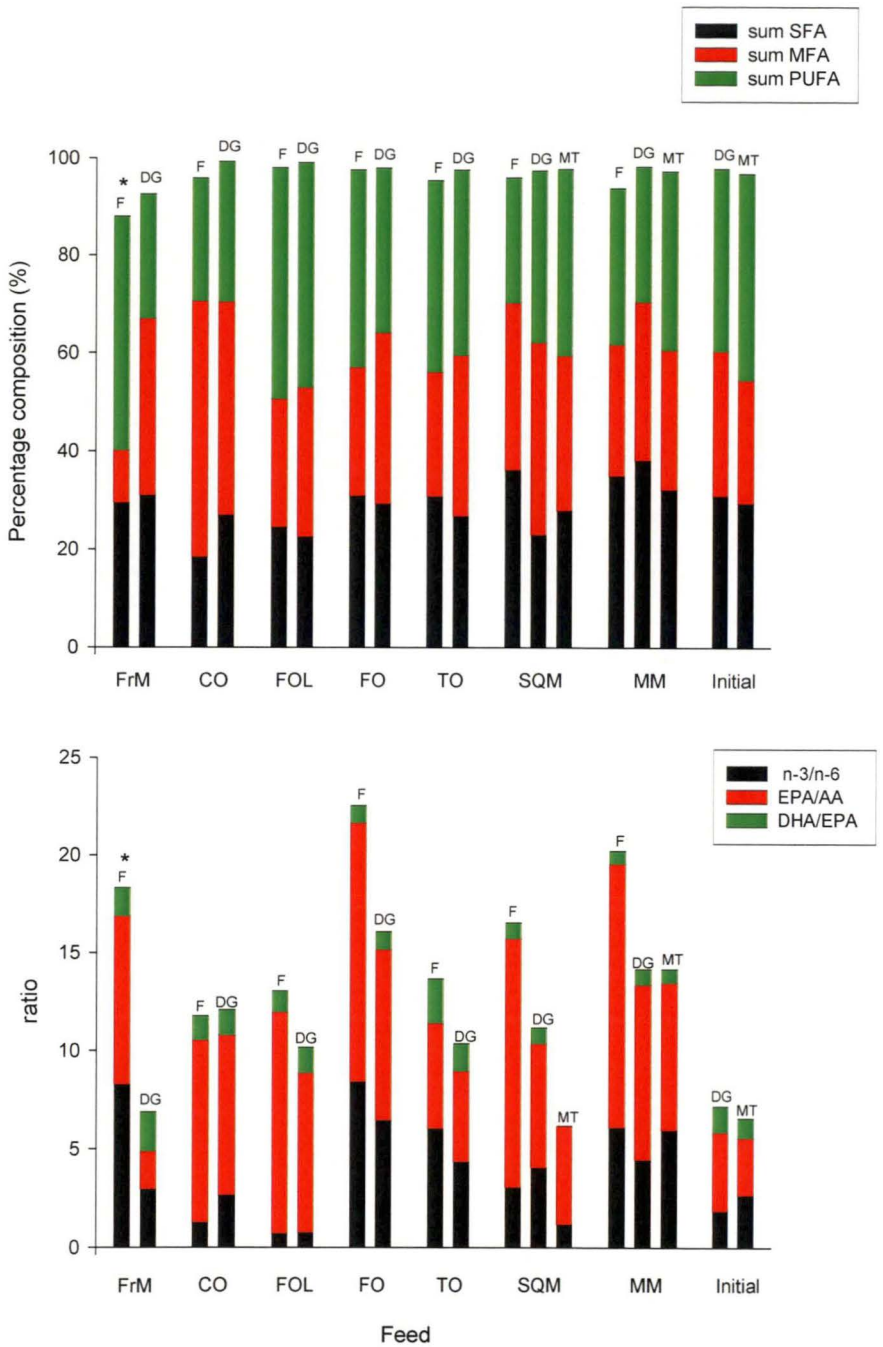

Figure 6.3. The major fatty acids (A) and essential fatty acid ratios (B) as a percentage of the total fatty acids in the feed (F), digestive gland (DG) and muscle tissue (MT) of juvenile J. edwardsii after feeding for 10 weeks. Fresh mussel fed lobsters and initial compositions are included for reference. Lobsters (n=5) from within each formulated feed treatment (n=3) and fresh mussel feed (n=2) were pooled and analysed in duplicate for all samples. * Fresh mussel composition was calculated from the fatty acid profile from Murphy et al., (2002).

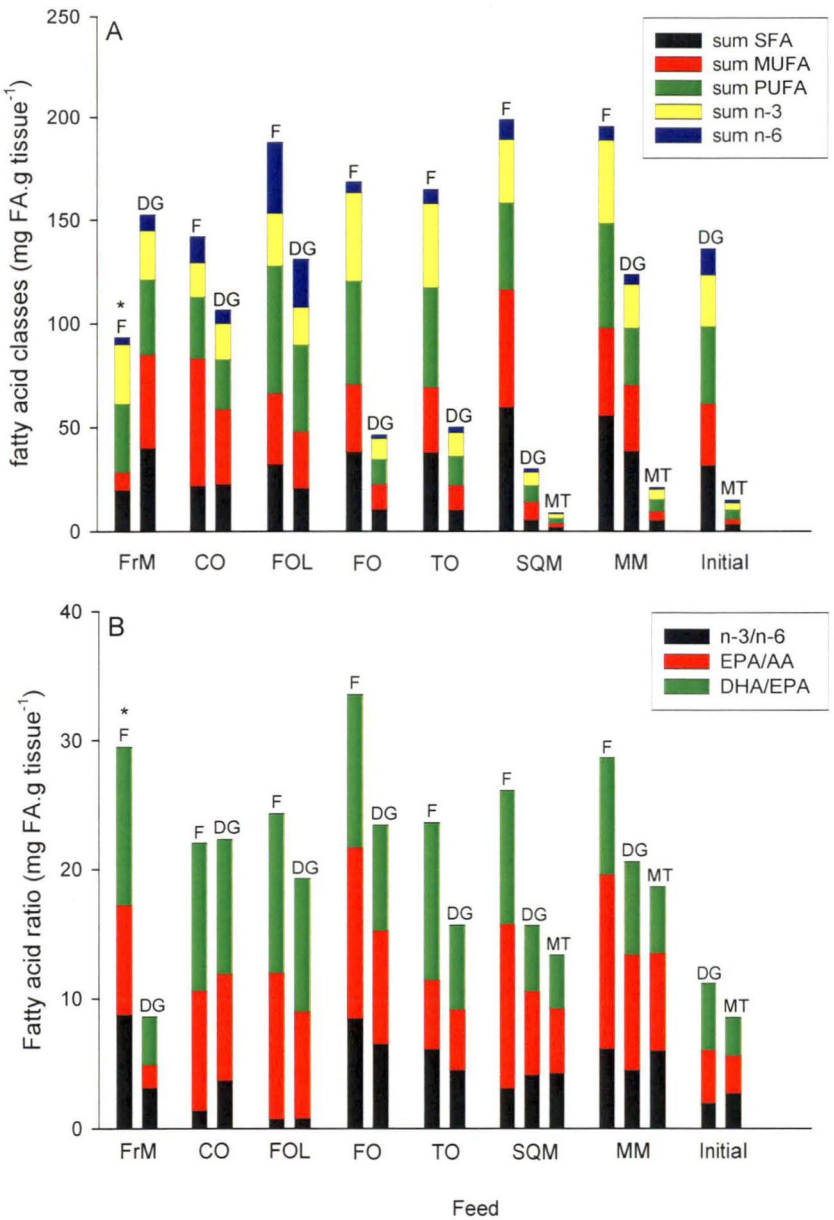

Figure 6.4. Quantitative summary of fatty acid (FA) classes (**A**) (mg FA.g wet tissue^{-1}) and essential fatty acid ratios (**B**) of muscle tissues from juvenile lobsters *Jasus edwardsii* fed feeds containing canola oil (CO), fish oil and lecithin (FOL), fish oil (FO), tuna oil (TO), squid meal (SQM), mussel meal (MM) and the reference feed fresh mussel (FrM) over a 10 week growth trial. Fresh mussel fed lobsters and initial compositions are included for reference. Lobsters (n=5) from within each formulated feed treatment (n=3) and fresh mussel feed (n=2) were pooled and analysed in duplicate for all samples. * Fresh mussel composition was calculated from the fatty acid profile from Murphy et al., (2002).

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Chapter 7

The application of nitrogen excretion and oxygen consumption measures to identify optimal protein/energy levels in juvenile lobster

7.1 Abstract

The efficient provision of energy in lobster feeds is of importance to further improve formulated feeds for the developing rock lobster aquaculture industry. The present study investigated the oxygen consumption, ammonia-N excretion and O:N ratio for juvenile *Jasus edwardsii* in order to determine the preferred energetic substrate. A closed respirometer system was investigated as a method to measure the metabolic responses of lobsters fed six isolipidic and isoenergetic formulated feeds containing 27 to 56% crude protein. Six lobsters were fed each feed and nitrogen excretion and oxygen consumption measured over the following 24 h period. There was a significant linear increase in oxygen consumption with increasing lobster weight, but no relationship between lobster weight and ammonia-N excretion. Ammonia-N excretion ranged between 0.103 – 0.293 $mgNH_3.gBW^{-1}$, and oxygen consumption between 2.72 – 3.24 $mgO_2.gBW^{-1}$. O:N ratios (11-32) indicated that *J. edwardsii* oxidise lipid in feeds containing low protein levels, and at high protein levels oxidise carbohydrates and proteins. The study suggests protein oxidation beyond the optimum digestible crude protein level is responsible for decreased growth rates at high protein levels.

7.2 Introduction

The metabolism of proteins (amino acids) as an energy source has been demonstrated for many aquatic species, but has not been directly investigated in *Jasus edwardsii.* The growth response of *J. edwardsii* to increasing protein levels has been shown to follow a bell-shaped curve (Ward et al., 2003), where at the lower and upper protein levels in feeds, the efficiency of growth is reduced. Similar patterns of protein utilisation have been observed in penaeid prawns (Alava and Lim, 1983; Hajra, 1988) and described an inefficient retention of synthesised proteins as growth, that was independent of feed intake and digestibility of the feeds. In teleosts, increasing protein/energy ratios beyond the optimum ratio stimulates an increase in protein synthesis, but a reduced synthesis retention efficiency (Carter and Houlihan, 2001).

The efficiency with which proteins are assimilated into new tissues is influenced by a variety of factors including the quality and quantity of protein available, the inclusion of alternative energy substrates in feeds and the efficiency of protein digestibility (Bureau et al., 2003; Carter and Houlihan, 2001; Wilson, 1989). Protein synthesis is directly related to the amino acid profile of ingested proteins, where amino acids in excess above the optimum relative amino acid ratio, are deaminated and excreted from the body of crustaceans in a range nitrogenous compounds, predominantly as ammonia-N, and to a lesser extent urea-N and amino acids (Carter and Houlihan, 2001; Regnault, 1987). Therefore, to formulate effective feeds, the provision of protein of optimal amino acid composition and level is necessary to optimise the retention of synthesised proteins (as growth), and to reduce protein degradation and excretion. Accordingly, the ingested nitrogen and magnitude of ammonia-N excretion can be used to assess the protein retention of feeds with varying protein quality and quantity.

The metabolism of amino acids, fatty acids and carbohydrates to their metabolic end-products, requires the consumption of oxygen in stoichiometric ratios (Brafield and Solomon, 1972). Consequently the ratio of oxygen consumption and ammonia-N excretion therefore can provide an indirect calorimetric ratio to determine the substrate

being respired. Few studies have measured oxygen consumption, ammonia-N excretion or O:N ratios in relation to feed composition in spiny lobsters (Perera et al., 2005). Most previous studies detail metabolic changes due to size, emersion and environmental change (Buesa, 1979; Crear and Forteath, 2000; Huang and Chen, 2001; Taylor and Waldron, 1997; Winget, 1969).

The amount of oxygen consumed during the process of food consumption and digestion, is described by the specific dynamic action (SDA). The magnitude of SDA is determined by the energy expended in processes including feed manipulation and handling, nutrient digestion and absorption, nutrient metabolism, the synthesis of new products and excretion of waste end-products (Jobling, 1994; Whiteley et al., 2001). Therefore the present study was designed to measure baseline ammonia-N excretion and oxygen consumption data for unfed animals and to measure SDA in fed lobsters (Dall, 1986). Feeds (isolipid and isoenergetic) containing an improved protein source, at a wide range of protein levels either side of the optimum (Ward et al., 2003) were formulated, and O:N ratios determined with the aim to identify the most effective protein level, and the energetic substrates respired under a range dietary protein/energy levels. Due to variable feed intake, the replication for these measurements was reduced and the dataset was modified prior to analysis to remove lobsters consuming sub-maintenance levels of protein.

7.3 Materials and methods

7.3.1 Experimental feeds

Feeds were made at the School of Aquaculture, University of Tasmania. Feeds were formulated to be isolipidic and isoenergetic, but to contain a range of protein levels (Table 7.1). The protein was made from a blend of South American fish meal (Skretting Australia, TAS) and mussel meal (National Institute of Water and Atmospheric Research, New Zealand) at a ratio of 5:1. These sources were selected based on apparent digestibility data, (Chapter 2), while limiting lipid levels for formulation. South

American fish oil (Skretting Australia, TAS) was the only oil source. Pre-gelatinised maize starch (BO11C, Sigma Chemicals, NSW) and 70% vital wheat gluten (Sigma Chemicals, NSW) were used to balance the energy and protein levels of the feeds, and bentonite (Sigma Chemicals, NSW) was added where necessary as an inert filler. A vitamin premix was made from individual vitamins (Sigma Chemicals, NSW) according to D'Abramo (1997), Stay C vitamin C and carophyll pink (Roche Australia, NSW), were included with the antioxidant Banox E (Sigma Chemicals, NSW). The alginate binder Manucol DM (Geraldton Industries, NSW) was combined with trisodiumpyrophosphate (TSPP) (Ajax Chemicals, NSW) to avoid premature calcium binding until the final pellet form was achieved.

The dry materials were thoroughly mixed in a Hobart mixer for 30 min. Vitamins and inert digestibility markers were included into smaller batches and mixed well before addition to the remainder of the dry materials. Fish oil was added and mixed for 30 min, and then the mixture was transferred to an Italapast pasta maker. Distilled water was added at 400 ml water/kg and the moist mixture extruded through a 1 mm die, and cut automatically to 1.5 cm length pellets. The pellets were immersed in a 10% $CaCl_2$ bath for 30 seconds, then dried on racks in a fan forced oven at 25 °C for 24 h. Pellets were stored in airtight containers at -20 °C prior to use.

7.3.2 Experimental lobsters

Animals were obtained from the Marine Research Laboratories, TAFI, in Hobart where they had been ongrown over the proceeding months from puerulus caught in Jan 2001. Lobsters were maintained in a recirculating seawater system and fed a mixed diet of prawn pellets (Higashimaru, size 12) and freshly opened blue mussels (*Mytilus edulis*). Water parameters remained within recommended limits for crustaceans (Crear and Allen, 2002).

7.3.3 Experimental procedure

Eighteen intermoult lobsters (according to Turnbull, 1989) were randomly selected from the population stock, blotted dry, weighed, sexed and placed into 18 individual conical experimental tanks. Each tank had a 12 mm black mesh base, a hide and a feed tray. Aerated water was supplied through a surface inlet, and water left the tank through a standpipe, with water circulation provided by a power head pump fitted in each tank. Diets were assigned to tanks and fed for an acclimation period of 7 d during which lobsters were fed at 6:00 pm for 1 h. Uneaten feed was removed from the tank, and the tank flushed of any faeces and debris. On day 8, feeding was staggered by half an hour for three groups of 6 tanks, at 1800, 1830 and 1900 h. After one hour of feeding, the lobster and hide was gently removed from the tank, and placed inside a 19.8 L plastic tank containing 0.2 μm filtered seawater at 19°C, and fitted with an airtight bung ensuring no air bubbles were trapped inside. The tanks remained sealed for 12 h, after which time the lid was removed, triplicate 30 ml water samples for urea-N and ammonia-N analysis were taken and dissolved oxygen measured immediately (YSI 550DO Dissolved oxygen logger). The animal was carefully transferred to an identical new tank containing 100% oxygenated 0.2 μm filtered seawater. Lobsters remained motionless during transfer to the new tank. This process was repeated to measure oxygen consumption and ammonia-N excretion data over two consecutive 12 h periods. At the end of the 24 h sampling period, each lobster was removed from the tank, 6 further water samples, as at 12 h, were taken for ammonia-N and urea-N analysis. The lobster was removed from the tank, dried and weighed, then killed by immersion in an ice slurry. The digestive gland was dissected from the dead lobster, weighed and snap-frozen in liquid nitrogen. The abdominal muscle tissue was removed from the exoskeleton and snap-frozen in liquid nitrogen.

Blank water samples from three identical experimental tanks using the same source of water but without animals were taken at the end of each 12 h experimental period. Tanks containing faeces after a lobster was removed were resealed and water measurements taken over 12 h periods to assess the effect of microbial activity on oxygen consumption.

7.3.4 Chemical analyses

Nitrogen in feeds was determined by Kjeldahl digestion in H_2SO_4 with Cu/Ni catalyst, and liberated ammonia-N was measured using the Kjeldahl distillation unit, and titrated against 0.56 M HCl. Crude protein was calculated as nitrogen X 6.25. Energy was measured by complete combustion in a Gallenkamp bomb calorimeter. Total lipid was determined gravimetrically using a chloroform:methanol solvent extraction as modified from Bligh and Dyer, (1959). Ash was determined after combustion in a muffle furnace for 16 h at 550˚C. Carbohydrate was determined by difference of total dry weight from the sum of protein, total lipid and ash (AOAC, 1990).

Ammonia-N in water was determined according to the indophenol blue method. Water samples, reagent blanks and a standard curve were mixed with salicylate catalyst were incubated in an alkaline-hypochlorite solution in darkness for 1 h, then indophenol blue absorbance was read at 640 nm (Bower and Holm-Hansen, 1980). Urea-N was analysed by the method of (McCarthy, 1970) and water samples, reagent blanks and standard curves were boiled in the dark with reagents for 10 min then absorbance was read at 535 nm. Very low concentrations of urea-N were detected across both feed treatments and control animals. As there was no difference in urea-N levels between the control tanks and experimental tanks after 12 h of excretion, urea-N was considered negligible and omitted from further statistical analyses.

7.3.5 Calculations

The experiment was designed to measure changes in oxygen consumption, ammonia-N excretion and to derive O:N ratios for lobsters in response to changing dietary nutrient levels. However the low feed intake of feeds at high and low protein levels required the removal of non-feeding lobsters from the analyses. As feed intake was limited, apparent digestibility was not measured in these feeds, and nutrient digestibility was assumed to be similar between all feeds as shown previously for similar formulations (Ward, 2003). In order to determine a criteria upon which to exclude data, an independent data set was used from a growth trial where similar sized juvenile lobsters were fed feeds of comparable ingredient composition and nitrogen level (Ward et al., 2003). Nitrogen

intake.d^{-1} was plotted against productive protein value to determine an independent measure of the maintenance levels of ingested N per day required for juvenile *J. edwardsii*. Productive protein values were modelled against mg N intake per day, the regression explained by a quadratic polynomial ($y=-80x^2-8x-2.0047$, $R^2=0.885$). An approximation of maintenance levels was adopted where mg N intake produced no net gain of N growth, which occurred at 2mg N intake per day. Lobsters in the present study that consumed less than 2mg N per day were considered to have consumed sub-maintenance levels of N, and likely to produce a non-feeding metabolic response and were therefore excluded from analyses. The remaining lobsters that did not consume feed prior to the testing period formed the basal metabolism group (B.M.). The current dataset was divided into three groups – lobsters that ate more than 2 mg N (n=25), lobster that ate less than 2 mg N (n=1) and control lobsters that consumed no feed (n=9). The lobster that ate less than 2 mg N was omitted from calculations.

7.3.6 Statistical analysis

Mean (± standard error) responses to each feed treatment are presented unless stated otherwise. Residual plots of the data were assess the normality and homogeneity and no transformations were necessary. Variability between feed treatments was assessed using one-way ANOVA and where indicated by two-way ANOVA (SPSS statistical software v11.5). Metabolic responses of lobsters were examined using linear regression as indicated. Significance was accepted at $P<0.05$. As the size of juvenile lobsters ranged from 13.1 to 36.9 g, all measures were standardized against body weight.

Due to an oxygen probe malfunction during sampling, two outlying results from 27P and 33P were removed from oxygen analyses (and corresponding data in regressions against oxygen consumption, requiring equal datasets).

7.4 Results

7.4.1 Biological indices of experimental lobsters

Lobsters selected for experimentation were of similar whole body weight ($F_{5,19}$=1.10, P=0.339), digestive gland weight ($F_{5,19}$=1.80, P=0.161) and digestive gland index (DGI) ($F_{5,19}$=1.144, P=0.372) (Table 7.2). The sex of lobsters was not different among feed treatments ($F_{5,19}$=0.086, P=0.994). The experimental test period was not significantly different throughout the experiment ($F_{5,19}$=0.056, P=0.998). While there were significant differences in ammonia-N excretion between the 6 test groups ($F_{5,17}$=4.06, P=0.013), there were no differences between the two testing occasions ($F_{1,20}$=0.265, P=0.612) and the group differences were related to the deliberate section of individuals according to their feed consumption. The feed intake ($mg.gBW^{-1}$) of lobsters fed high (56P and 53P) dietary protein feeds were significantly less than 39P ($F_{5,19}$=4.578, P=0.007) (Table 7.3). Lobsters consumed more nitrogen ($mgN.gBW^{-1}$) when fed the 39P and 45P feeds ($F_{5,19}$=7.04, P=0.001) (Table 7.3).

7.4.2 Metabolic indices

The regression of total ammonia-N excretion (mg NH_3) against the weight of the lobster (g) was significant, but not a strong relationship (R^2=0.26) (Fig. 1) indicating that the majority of the variation (74%) in the ammonia-N excretion rates was not due to animal weight. However, there was a strong significant relationship between the weight of lobsters and their total oxygen consumption (mg O_2) (Fig. 2) (R^2=0.76). All ammonia and oxygen measurements are expressed as mg per g body weight. There was no significant increase of ammonia-N excretion ($mgNH_3.gBW^{-1}$) ($F_{5,17}$=2.39 P=0.082) or the oxygen consumption ($mgO_2.gBW^{-1}$) ($F_{5,17}$=1.94, P=0.140) with increasing nitrogen content of feeds (Table 3). The variation in ammonia-N excretions measured was not related to differences in feed intake (R^2=0.093) or nitrogen intake (R^2=0.011). Likewise, neither feed intake (R^2=0.009) or N intake (R^2=0.002) explained changes in oxygen consumption. There was a linear relationship between the oxygen consumed by lobsters and the amount of ammonia-N excreted (Fig. 3), however this relationship (R^2=0.28) accounted for only a small portion of the variation in measurements. There was no

relationship between the O:N ratio and the protein levels in feeds (R^2=0.158), and O:N did not correlate with any of the biological parameters measured.

7.5 Discussion

7.5.1 Nitrogen excretion and oxygen consumption

Very little data describing changes in metabolism with feed formulation are available for spiny lobsters, and *J. edwardsii*. Some nitrogen excretion data associated with handling and emersion of *J. edwardsii* exists (Crear and Forteath, 2000; Morris and Oliver, 1999; Taylor and Waldron, 1997) and a few studies have investigated oxygen consumption of lobsters fed natural feeds (Crear and Forteath, 2000; Lefever, 2000), and of formulated feeds in *Panulirus argus* (Perera et al., 2005). Nitrogen excretion in lobsters is affected by a variety of factors. Feed typically has the strongest influence on ammonia-N excretion (Crear and Forteath, 2002). However, in the present study, while ammonia-N excretion tended to increase with increasing protein level, there was no significant relationship to feed protein level, or amount of ingested protein. Perera et al., (2005) fed *P. argus* a feed similar to that in the present study, composed of fish meal and clam meal (at approximately 5:1 respectively), and reported mean ammonia-N excretion at 25% protein of 0.192 $mgNH_3.gBW^{-1}$, and at 35% protein, 0.216 $mgNH_3.gBW^{-1}$, which was within with the range in the present study 0.103 to 0.298 $mgNH_3.gBW^{-1}$. The optimum protein level in juvenile *J.* edwardsii was previously determined at 31 %DCP at 9% lipid (Ward et al., 2003), which corresponds to 42% crude protein (assuming 74% protein digestibility of a fish meal reference feed, Chapter 2). Between 39P and 45P, ammonia-N excretion appeared to increase in the present study, and with further data, may explain previous decreases in growth above the optimum protein level (Ward et al., 2003). The excretion of ammonia-N was not related strongly to lobster size which is most likely due to the narrow size range, and differing feeds fed to the lobsters tested.

The consumption of oxygen in the 24 h post feeding was not significantly related to the composition of the feed, nor the weight of feed or nitrogen ingested. Oxygen

consumption was related to body weight, which is similar to that seen in previous studies of *J. edwardsii* adults (Crear and Forteath, 2000) and juveniles (Lefever, 2000) and other decapod crustaceans (Bridges and Brand, 1980). The average oxygen consumption over 24 h in the present study (2.55 to 3.38 $mgO_2.gBW^{-1}$) was similar to previous studies in juvenile *J. edwardsii* fed fresh mussels and adult *J. edwardsii* (3.84 $mgO_2.gBW^{-1}$) (Crear, 1998), and slightly higher than *P. cygnus* 2.26 $mgO_2.gBW^{-1}$ (Crear and Forteath, 2001). When lobsters were placed in respirometers, they remained motionless, clinging to hides place in the vessel. Periodic checks indicated that lobster movement in the respirometer was minimal, therefore it was assumed there was no active movement and the unfed lobster oxygen consumption was representative of the basal metabolism (Dall, 1986). The average resting metabolic rate in the present study was 2.95 $mgO_2.gBW^{-1}$ and was similar to resting oxygen consumption rates previously determined for juvenile *J. edwardsii* (1.35 to 2.57) (Lefever, 2000), but was higher than rates determined for adult *J. edwardsii* (0.96) (Crear and Forteath, 2000) and for *P. argus* (0.96 to 1.56) (Perera et al., 2005). The basal metabolism in the present study was elevated above the response of fed lobsters, therefore was not an accurate measure and was not used in intended calculation of SDA.

7.5.2 Metabolism in lobsters

The ratio of consumed oxygen and excreted nitrogen corresponds to the oxidation of energetic substrates, where ratios below 7 indicate protein oxidation, 7 to 24 for carbohydrate and over 24 indicating predominately lipid oxidation (Mayzaud and Conover, 1988). The O:N ratios in the present study were lowest for low carbohydrate/high protein feeds, intermediate in feed 56P (11), and increased in feeds with high carbohydrate/low protein in feed 27P (32), suggesting a shift from lipid oxidation at low protein (high carbohydrate), to protein-carbohydrate oxidation in high protein (low carbohydrate) feeds. This suggests a reduced ability to utilise high carbohydrate levels in feeds. Similar O:N ratios were reported in the spiny lobster, *P. argus* fed fish meal based feeds at 25% (40) and 35% (10-15) (Perera et al., 2005). *M. rosenbergii* fed low carbohydrate/high protein feeds produced O:N ratios of 11 and 13 (40 and 49% protein) and high carbohydrate/low protein feeds (23 and 32% protein)

produced O:N ratios of 23 and 21 (Millikin et al., 1980). Similar O:N ratios were seen in juvenile *H. americanus;* 16 for feeds with 23% protein and 13 at 51% protein. Increasing O:N with decreasing protein/increasing carbohydrate has been observed in several species; *Homarus americanus* (8 to 11) (Koshio et al., 1992), *Macrobrachium rosenbergii* 20 to 49 (Gonzalez-Pena and Blumer Soares Moriera, 2003). In general, increasing dietary protein leads to an increase in protein oxidation, however there is inter-species variation in the preferential oxidation of lipid or carbohydrate at low protein levels.

7.5.3 Conclusions

The present study confirms that *J. edwardsii* utilise lipid as an energetic substrate, but at high protein levels will oxidise proteins for energy. The ability of *J.* edwardsii to utilise starch, especially at higher inclusion levels, was a complicating factor in interpreting the results. However, the trend toward increased ammonia-N excretion above the optimum protein level, may partly explain previous decreases in growth at high protein levels, due to increased costs associated with high amino acid intake and oxidation. The calculation of SDA in the present study was not possible due to and elevated measure of basal metabolism, and differences in metabolic response between feeds were masked by high variation between lobsters. Causes of variation in metabolic responses are largely undefined, and make precise bioenergetic measures in crustaceans challenging. Ensuring an accurate basal metabolic measure is crucial to the calculation of SDA, and the cause for the elevation may be due to a range of factors including individual variation, stress, nocturnal activity or increased foraging in unfed animals. The true measurement of SDA in crustaceans is difficult, as although stationary, crustaceans continually move maxillipeds and maxillae and preen appendages. The combined use of infrared video footage with respirometers would be of advantage to control data for excessive physical movement. If respirometry is to be used, longer term experiments would be required for *J. edwardsii.* These would be relatively complex and would argue in favour of growth trials providing more useful data at this stage in determining practical information for *J. edwardsii.*

Table 7.1. Feed ingredients and chemical composition ($g.kg^{-1}$).

Ingredient	***27P***	***33P***	***39P***	***45P***	***53P***	***56P***
Fish meal	333.33	416.67	500	583.33	666.67	716.67
Mussel meal	66.67	83.33	100	116.67	133.33	143.33
BO11C starch	425	330	231	145	50	0
Bentonite	22	29	37.9	36.3	41.8	40.8
Fish oil	69	58	49	37.5	28	19.5
Vitamin premix	1.9	1.9	1.9	1.9	1.9	1.9
Vitamin A	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin E	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin C	1	1	1	1	1	1
Mineral premix	0.5	0.5	0.5	0.5	0.5	0.5
Manucol	60	60	60	60	60	60
TSP Phosphate	20	20	20	20	20	20
Cholesterol	2	2	2	2	2	2
Banox E	0.2	0.2	0.2	0.2	0.2	0.2
Yttrium	1	1	1	1	1	1
Ytterbium	1	1	1	1	1	1
Chemical composition $g.kg^{-1}$						
Crude protein	265.9	330.2	386.8	448.4	531.7	559.1
Crude lipid	126.5	124.8	122.1	123.7	129.1	124.9
Gross energy	16.87	17.11	17.14	17.24	17.34	17.30
CHO	468.3	387.0	321.6	243.2	134.4	99.4
Ash	139.3	158.0	169.5	184.7	204.8	216.6
Moisture	63.7	38.2	50.1	55.0	81.6	63.8

Table 7.2. Biological parameters (mean ± S.E.) of juvenile *J. edwardsii* that were fed feeds containing 27-56% protein, and an unfed group of lobsters (B.M.).

		27P	***33P***	***39P***	***45P***	***53P***	***56P***	***B.M.***[2]	***F***	***P***
	Units									
Number of lobsters		5	6	6	3	3	2	9		
Test period	h	24.13	23.96	23.92	24.08	23.83	23.92	24.26	0.06	0.998
S.E.		0.4	0.4	0.4	0.5	0.5	0.4	0.3		
Live weight	g	24.67	26.60	24.01	31.06	29.49	31.85	23.09	1.10	0.393
S.E.		3.4	2.2	2.3	2.6	3.0	5.0	2.9		
DG weight	g	1.28	1.30	1.31	1.89	1.82	1.88	1.04	1.80	0.161
S.E.		0.2	0.2	0.1	0.2	0.3	0.1	0.2		
DGI[1]	%	5.13	4.80	5.49	6.07	6.08	6.01	4.3	1.14	0.372
S.E.		0.6	0.5	0.1	0.4	0.5	0.7	0.6		

[1]Digestive gland index (DGI) = wt DG/wt WB*100

[2]Basal metabolism (BM) = lobsters that consumed no feed, not included in one-way ANOVA.

Table 7.3. Metabolic performance data (mean ± S.E.) of juvenile *J edwardsii* over a 24 h period after consuming feeds containing a range of protein levels (27-56%), and of unfed animals (B.M.)

		27P	***33P***	***39P***	***45P***	***53P***	***56P***	***B.M.***[1]	***F***	***P***
Metabolic indices	*Units*									
Number of lobsters		5	6	6	3	3	2	9		
Feed intake	$mg.gBW^{-1}$	11.19^{ab}	16.46^{ab}	29.32^{b}	22.72^{ab}	7.03^{a}	7.18^{a}	0	4.58	**0.007**
S.E.		3.6	5.4	2.6	2.1	0.8	2.2	0		
Nitrogen intake	$mg.gBW^{-1}$	0.476^{a}	0.870^{ab}	1.815^{b}	1.630^{b}	0.598^{a}	0.643^{a}	0	7.04	**0.001**
S.E.		0.15	0.29	0.16	0.15	0.07	0.20	0		
Ammonia-N excretion	$mgNH_3\text{-}N.gBW^{-1}$	0.103	0.133	0.126	0.209	0.206	0.293	0.149	2.39	0.082
S.E.		0.02	0.04	0.03	0.06	0.07	0.00	0.031		
Oxygen consumption	$mgO_2.gBW^{-1}$	2.72	2.55	2.65	3.38	3.03	3.24	2.95	1.94	0.140
S.E.		0.4	0.3	0.2	0.0	0.2	0.1	0.5		
O:N		32.20	29.43	28.99	22.10	20.65	11.07	26.96	0.81	0.559
S.E.		7.2	8.2	8.5	9.7	9.5	0.4	9.31		

Bold case indicates significant ANOVA result. Similar superscripts indicate no significant difference.

[1]Basal metabolism (BM) = unfed lobsters, not included in one-way ANOVA.

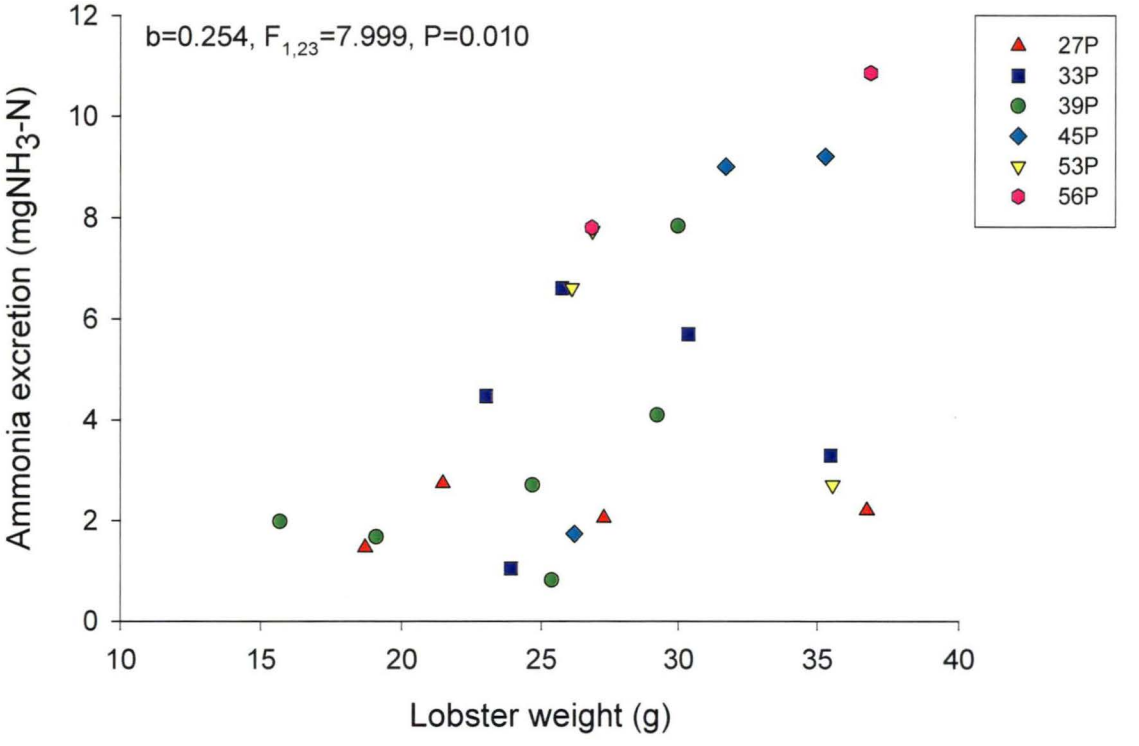

Figure 7.1. Regression of ammonia-N excretion (mg NH_3-N) and lobster weight of juvenile *J. edwardsii* over a 24 h period after consuming feeds ranging in crude protein content between 27-56%. (Two outliers removed from data set from 33P and 27P).

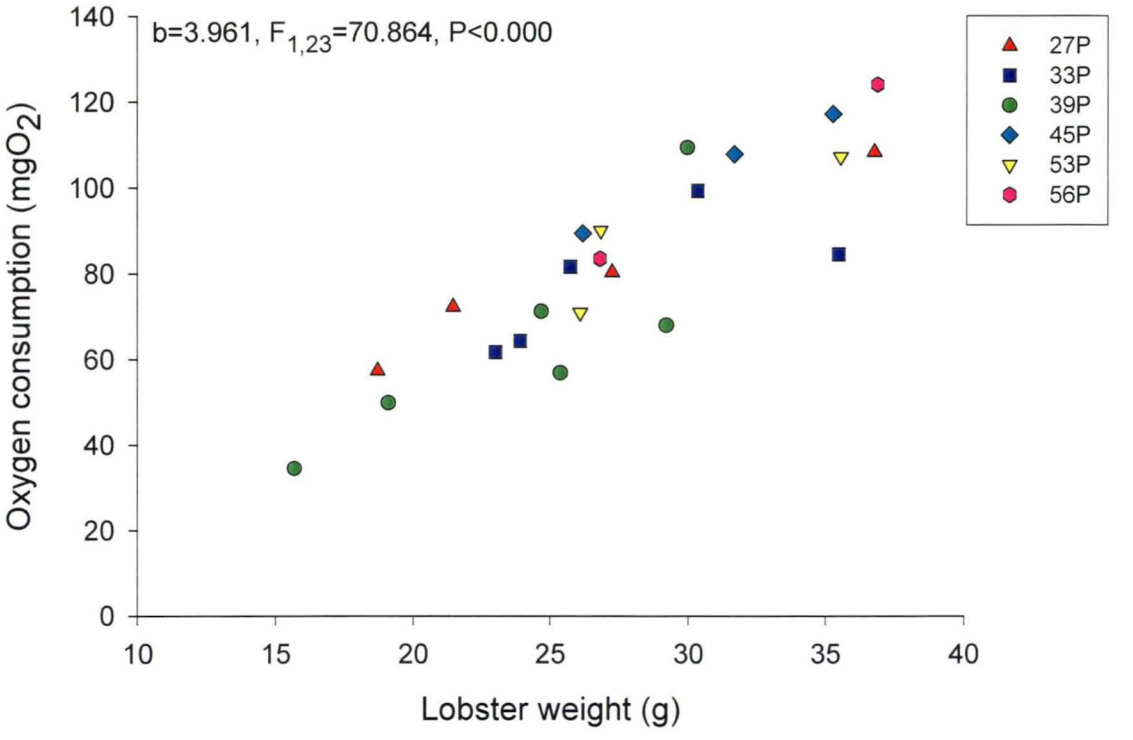

Figure 7.2. Regression of oxygen consumption (mg O_2) and total body weight of juvenile *J. edwardsii* over a 24 h period after consuming feeds ranging in crude protein content between 27-56%. (Two outliers removed from data set from 33P and 27P).

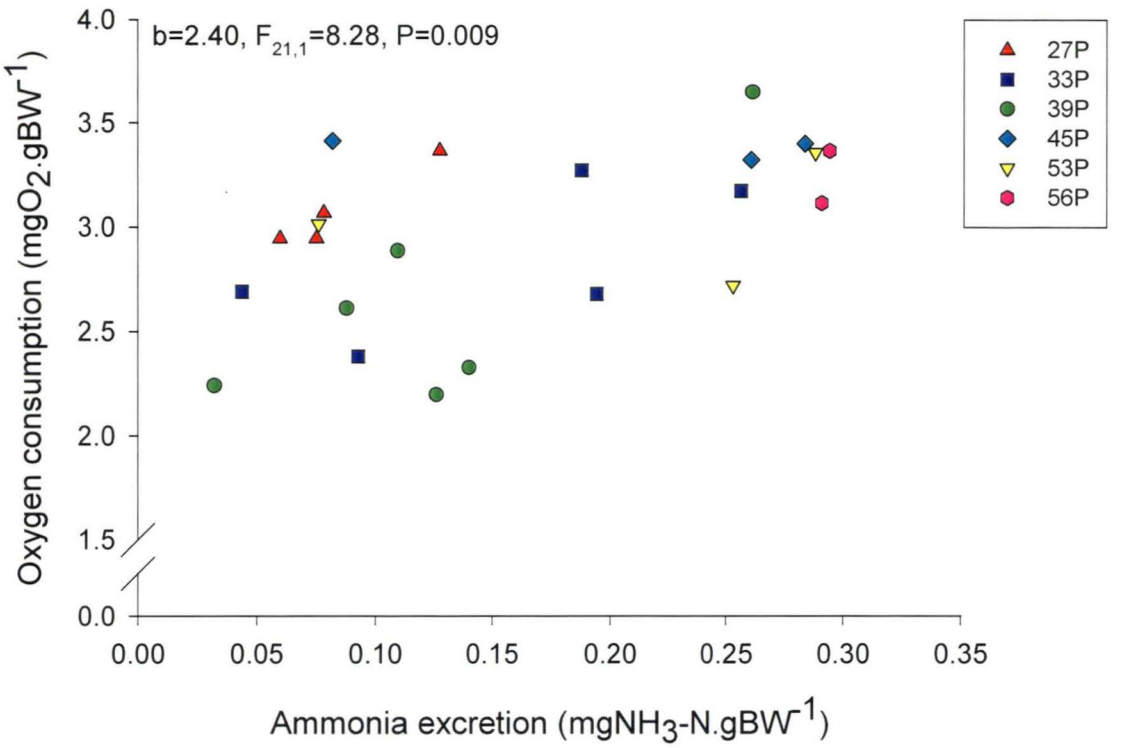

Figure 7.3. Total oxygen consumption ($mg.gBW^{-1}$) according to total ammonia-N excretion ($mgNH_3.gBW^{-1}$) in juvenile *J. edwardsii* over a 24 h period after consuming feeds ranging in crude protein content between 27-56%. (Two outliers removed from data set from 33P and 27P)

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Chapter 8

General Discussion

8.1 Overview and key findings of this study

There is significant interest in the aquaculture of *Jasus edwardsii* in Australia and New Zealand, and the economic feasibility of commercial lobster aquaculture requires the development of formulated feeds (Crear et al., 1998). Current knowledge of the nutrition of *J. edwardsii* is limited to a few studies, therefore the present study was developed to further our knowledge of lobster nutrition, methods for determining nutritional requirements in lobsters, and to provide practical data with relevance for future research and commercial feed production. Some key findings of this study include:

Protein digestibility

- Apparent protein digestibility from day and night-time feeding did not differ, and apparent protein digestibility did not change over 3 weeks
- Yttrium oxide provided lower estimates of apparent protein digestibility than ytterbium acetate
- Fish meal was not well digested and values were similar to soybean meal
- Digestibility of finely processed plant meals was high
- Mussel and prawn meals were highly digested by lobsters

Dietary lipids, tissue composition and growth

- The inclusion of plant oil sources did not affect the growth rate of lobsters
- A combination of lecithin with fish oil, increased the abundance of lipid droplets in the digestive gland
- There are significant correlations between the composition of lipid classes in feeds and tissues with nutrient retention, nutrient efficiency and the storage of lipids in the digestive gland
- The fatty acid composition of lobster digestive gland reflected that of the feed
- Dietary phospholipid was converted to triglyceride and stored in lipid droplets in the digestive gland in fast growing lobsters
- Lobsters with poor growth had high proportions of phospholipid and sterol, and low triglyceride

- Histology of digestive glands was related to feeds, and lipid storage closely related to biochemical measurements of triglycerides

Lobster metabolism

- Lobsters metabolise lipids in low protein feeds, and carbohydrates and protein in high protein feeds
- Protein metabolism in high protein feeds may explain reduced growth rates in *J. edwardsii*

8.2 Standardising methods for nutritional research in lobster

As aquaculture grows to include new species, including *J. edwardsii,* there is an increasing need to adopt standardised approaches to nutrition studies. Comparison of results with those reported in the crustacean nutrition literature is often difficult due to differences in experimental protocol, analytical techniques and approaches. The challenges of defining nutritional parameters for spiny lobsters are well documented (outlined in chapter 1). Some of the factors complicating crustacean nutrition in contrast to teleost nutrition include discontinuous growth rates, energy expenditure and mobilisation associated with ecdysis, manipulative feeding and slow growth rates, which require adaptation of nutrition methods accordingly. In addressing research objectives, it is important to ensure the application to commercial farming situations. Much of the requirement determination studies in the literature use purified protein sources and do not assess the performance of commercial ingredients (Tacon, 1996). Several laboratories have established a standard reference protein for homarid lobsters, spiny lobsters and crabs (Boghen et al., 1982; Castell et al., 1989a; Castell et al., 1989b; Reed and D'Abramo, 1989; Ackefors et al., 1992; Lellis, 1992) and more recently in prawns (Glencross et al., 1999), however the adoption of these reference feeds has not widely occurred due to poor growth performance and availability of ingredients locally (Tacon, 1996). The successful use of any formulated feed to determine nutritional requirements in crustaceans requires minimal leaching of nutrients prior to ingestion, strong attractants to aid the location of feed, and improved nutritional profile to improve nutrient digestibility and avoid nutrient deficiencies across a wide range of species.

The assessment of feed performance is generally based on direct measures of lobster growth such as weight gain or instantaneous rates e.g. SGR, IGR, and as feed efficiency e.g. protein efficiency, productive protein values. In interpreting the performance of feeds, it is also important to consider the lobsters health and condition after long-term feeding and marketability e.g. colouration, taste and nutritional value to consumers. The application of histological assessment in addition to growth experiments provided further understanding of the processes affecting lipid deposition in the digestive gland.

The methods used to measure apparent digestibility differ widely. Researchers generally measure either the ingredient digestibility at $300g.kg^{-1}$ of a reference feed, or the digestibility of a compound feed (Lee and Lawrence, 1997; Smith and Tabrett, 2004). While ingredient digestibility provides a useful screening tool to identify readily utilised ingredients, the digestibility of the compound feed is also important to understand interactions between ingredients. The present study was designed as a screening method to select suitable protein sources for future experimentation in lobster, and identified that the digestibility of the current protein source, fish meal, was lower than expected, and the nitrogen assimilation from lobster feeds could potentially be improved by partial replacement or total replacement with mussel meal, prawn meal to improve feed AD_{CP} Further improvement to the commercial application of ingredient AD_{CP} may be achieved by determining AD_{CP} for a range of commercially used particle sizes, and the use of larger lobsters (producing more faeces) to increase sample sizes and replication. Designing novel faecal collections systems has improved digestibility measurement in tropical spiny lobsters (Irvin and Tabrett, 2005) where problems associated with leaching are greatly enhanced compared to temperate spiny lobsters. A recent study of methods to accurately measure digestibility in prawns outlines relevant methods that should be adapted for future lobster digestibility research (Smith and Tabrett, 2004), and reiterates the lack of detailed studies into the most appropriate inert markers for use in crustacean digestibility studies.

The application of bioenergetic studies to lobster nutrition was investigated. Very few studies have investigated the metabolism of energy substrates to define nutritional requirements in spiny lobster. While the assessment of basal metabolism was not

successful using the current system, the ratios of O:N metabolism indicated similar trends in substrate metabolism to previous studies in lobsters (Perera et al., 2005) and there is considerable potential to refine this method further and improve precision to measure SDA and in determine metabolic changes between nutritional regimes.

8.3 The ingredients for success in lobster feeds

The success of a lobster feed depends on the adequate provision of dietary ingredients that are effectively utilised for growth and meet the energetic demands of physiological processes. Ingredients available to aquafeed producers are subject to availability and more recently price. The increasing cost and progressive depletion of fish products has prompted much recent research to identify alternate protein and oil sources for aquafeeds to maintain the economic and environmental sustainability of aquaculture (Jensen and Alsted, 1990; Bell, 1998; Carter et al., 2003). Current commercial lobster farms in Vietnam and India rely on the feeding of trash fish (K. Williams, CSIRO Cleveland, personal communication), which is of unreliable supply and quality. Both existing lobster farms overseas, and future intensive lobsters farms in Australia will benefit from the production of an effective pelleted lobster feed. Commercial lobster feeds will require ingredients that are both readily available and highly utilised for growth. This study has highlighted the need to improve the current fish meal and fish oil feeds currently used for lobsters, and has provided information into the utilisation of dietary protein, lipid and lobster metabolism.

The protein in fish meal was not well digested (63%), and the partial (or full) replacement of fish meal with more digestible protein sources like mussel meal (98%) and prawn meal (77%) may improve nutrient availability in feeds. Protein from plant meals including soybean meal (61%) were digested with similar AD_{CP} to fishmeal indicating the potential to replace fish meal with soybean meal in lobster feeds. SQM was poorly digested, and produced poor growth rates and lipid deposition in the growth trial. Commercial feed producers include squid at low levels (2%) as an attractant and research has associated a growth-promoting effect, and increase in digestive enzyme activity has been associated with its inclusion (Cuzon et al., 1994; Cordova-Murueta and Garcia-Carreno, 2002; Perera et al., 2005). The poor digestibility and growth response of

lobsters fed SQM in the present study may have been associated with high saturated fatty acid levels (Merican and Shim, 1994), and potential oxidation of the meal as indicated by high free fatty acid levels (Sasaki and Capuzzo, 1984). The reported increase of digestive activity in lobsters fed squid meal feeds may explain the B-cell (and digestive vacuole) abundance in the digestive glands in the present study, where there appeared to be increased focus of cellular activity toward digestion, rather than energy storage.

The provision of lipid from fish oil and fish meal (FO) feeds produced mid-range growth rates. The inclusion of plant oils (canola oil and soybean lecithin) did not significantly affect growth rates of lobsters, and the inclusion of soybean lecithin with fish oil and fish meal (FOL), both maintained good rates and improved the lipid droplet deposition in the digestive gland compared to FO. The combination of plant oil and fish meal provided adequate essential fatty acids for growth derived from the marine meal, and the high levels of docosahexaenoic acid (DHA) in tuna oil feeds did not significantly improve the growth performance of these lobsters. Mussel and fresh mussel contributed high phospholipid content that was related to increased productive protein value and lipid droplet deposition in the digestive gland. Lipid droplet accumulation occurred in lobsters with high digestive gland triglyceride levels and the importance of dietary phospholipid sources, to improve triglyceride deposition in the digestive gland was directly related to growth.

The metabolism of *J. edwardsii* fed an improved protein source containing a combination of fish meal and mussel meal, indicated that in low protein feeds, lipid is metabolised, and at increasing protein level content, a combination of carbohydrate and protein is metabolised for energy. Above the optimum protein level there was an increase in ammonia excretion which may correspond to the decreases in growth at high protein levels observed previously in *J. edwardsii* (Ward et al., 2003).

8.4 Application of these findings to future lobster feeds

The experiments were designed with the intention for application of information to industry, and the present study expands the presently limited understanding of the spiny lobster nutrition. It appears that substantial replacement of fish products with plant oils

and meals will be possible in lobster feeds, and the essential fatty acid (EFA) requirements were met by the inclusion of fish meal with canola oil in feeds. However, it is not known to what level this replacement may extend before EFA (and essential amino acids) become limiting. From the present study it appears the best growth and digestibility occurred where mussel meal replaced fish meal and for the oils where there soybean lecithin was included with fish oil. The potential to include dietary PL to improve the utilisation of oils containing high levels of TAG has been reported previously in prawns (Teshima et al., 1986). Subsequent determinations of protein requirements using new protein sources (and amino acid profile) will be necessary. It is anticipated that over supply of protein in lobster feeds will produce high PPV (Ward et al., 2003) due to an increased metabolism of proteins for energy as seen in the present study and in *P. esculentus* (Hewitt, 1992). Tissue fatty acid profiles reflect the dietary fatty acid profile, and ensuring consumer acceptance of changed n-3/n-6 ratios and potential changes in appearance and taste of lobsters will be necessary (Nelson et al., In press) .

8.5 Future research

A close relationship between dietary phospholipid inclusion and mobilisation of lipids has been demonstrated, however, the role of dietary phospholipids in promoting growth is still unclear. There may be potential to incorporate dietary PL with poorly mobilised lipids to improve their energetic value and further study in this area is warranted. The role of nutrition on the formation and depletion of reserve cells in the digestive gland is of interest to optimise energy reserves for growth. Determining changes in the metabolism of lobsters provided with alternative ingredients may provide further insight into the influence of protein quality and level on the retention of dietary protein. Determining protein and energetic requirements across a range of lobster sizes will provide valuable insight to ontogenetic nutritional changes.

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